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EFFECTS OF REFRIGERATION UPON THE LARVÆ OF TRICHINELLA SPIRALIS

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INTRODUCTION

Prior to recent investigations, the first of which were briefly reported in a short article which appeared about two years ago (Ransom, 1914), it had been generally accepted as an established fact that the larvæ of *Trichinella spiralis* are very resistant to cold and that they survive exposure to temperatures much below the freezing point of water. In the article referred to, however, it was shown that former ideas concerning the resistance of trichinæ to cold were erroneous, and that as a matter of fact low temperatures have a very pronounced effect upon the vitality of these parasites. As a precise knowledge of the effects of refrigeration upon trichinæ is of considerable importance, an extended investigation has been made, the results of which are recorded in the present paper.

HISTORICAL SUMMARY

The following summary covers all of the published reports of experimental work on the effects of cold upon trichinæ so far as they could be traced in the literature.

Leuckart (1863a, p. 120) states that trichinæ are in the highest degree resistant to cold. He exposed some trichinous meat outdoors during cold January weather (−16° to −20° R.; −4° to −13° F.; −20° to −25° C.) for three days and nights. After thawing the meat, he fed it to a rabbit, which died a month later and was found to be very heavily infested with trichinæ. In another publication (1866a, p. 91) Leuckart notes that the place in which this meat was kept was somewhat protected, and it may therefore be presumed that the temperature to which the meat was actually exposed was probably not as low as indicated by the figures given. Leuckart remarks, however, that the meat was solidly frozen throughout.

Fiedler (1864, p. 466) exposed the leg of a trichinous rabbit to an outdoor temperature of −15° to −17° R. (−1.75° to −6.25° F.; −18.75°

to −21.25° C.) from January 6, 5 p. m., to January 7, 8 a. m.—i. e., for 15 hours. Examined on a warm stage, the trichinæ showed no movement. Some of the meat was fed to two rabbits on January 7, and on February 7, a month later, the rabbits were killed. In one of them a few encysted trichinæ were found. On January 16 he fed two rabbits with some trichinous meat which had been cut in fine pieces and exposed for 18 hours to a temperature of −11° to −12° R. (7.25° to 5° F.; −13.75° to −15° C.). On February 14 the rabbits were killed and carefully examined. No trichinæ were found.

Rupprecht (1864a) exposed trichinous meat during one night to an outdoor temperature of −18° R. (−8.5° F.; −22.5° C.) and found that the vitality of the trichinæ was not affected.

Kühn (1865b), according to Leuckart (1866a, p. 91), found that trichinous meat kept in an ice chamber for 1½ months was still infectious and that the trichinæ had lost their vitality only after the meat had been kept for 2 months in the ice chamber, the temperature of which was not given.

Gibier and Bouley (1882a) exposed some trichinous ham for 4 hours to temperatures of −27° C. (−16.6° F.) and −20° C. (−4° F.). In the first case the interior temperature reached −20° C. (−4° F.) and in the second −15° C. (5° F.). All of the trichinæ were found to be dead. They showed no movement when warmed, and they stained in a few minutes with anilin blue, methyl-anilin violet, and picrocarminate of ammonia. Some of the meat which had been frozen was fed during 8 days to five birds, which when examined later showed no trichinæ in the intestine; nor had any been found in the feces. Trichinæ from portions of the ham which had not been frozen were active when warmed to 40° C. and remained transparent and colorless for several days in staining solutions. Five birds of the same kind and age as those to which the frozen meat had been fed were similarly fed with the ham which had not been frozen, and large numbers of trichinæ were afterwards found in the feces and intestines.

These experiments of Gibier and Bouley seemed to show pretty clearly the destructive effects of low temperatures upon trichinæ, but later Gibier (1889a) came to the opinion that the death of the parasites was to be explained on the ground that they had already suffered a reduction in vitality from the action of salt, and, hence, readily succumbed to freezing. This opinion was based on the results of an experiment in which he exposed small fragments of fresh trichinous pork for 2 hours to a temperature of −20° to −25° C. (−4° to −13° F.). The parasites, when afterwards examined on a warm stage, were found to have lost none of their activity.

From the foregoing it would appear that the usual statements found in articles relating to *Trichinella spiralis* as to the resistance of this parasite to low temperatures have their principal basis in Leuckart's

single experiment, to which may be added, as supplementary support, Fiedler's first experiment, Rupprecht's experiment, and Gibier's experiment, a total of four experiments. Kühn's experiment perhaps has been considered as affording additional supporting evidence. The results of Fiedler's second experiment do not offset the results of his other experiment, nor those of Leuckart's and Gibier's experiments, as the failure to get an infestation in the two rabbits which were fed meat exposed for 18 hours to a temperature of 7.25° to 5° F. might have been brought about by something else than low temperature. Likewise, the results of Gibier and Bouley, when compared with those of Leuckart, Fiedler, and Gibier, tend to show only that trichinae are sometimes killed when exposed for a short time to temperatures below zero. The later explanation by Gibier (1889) that the trichinæ used in these experiments had lost so much vitality on account of previous salting of the meat that they succumbed, whereas they would not have done so if the meat had been fresh, has been accepted by those authors who have mentioned Gibier and Bouley's work. It should be noted, however, that in the experiment upon which Gibier (1889) based his explanation of the results of the earlier experiments by himself and Bouley the meat was exposed for only 2 hours as compared with 4 hours in the earlier experiments.

So far as appears in the available literature, after the later experiments conducted by Gibier (1889), no further work on the effects of cold upon trichinæ was done until the investigations undertaken by the present writer, 25 years later, the first of which were recorded briefly in an article (Ransom, 1914) already mentioned.

A few additional data gathered in these investigations were given in a later paper (Ransom, 1915).

Recently Schmidt, Ponomarer, and Savelier (1915) have published a preliminary report of some investigations of the effects of cold upon trichinæ in which they state that a long series of experiments has led to the following results:

1. A temperature of 0° C. (32° F.) has no influence upon the vitality of encysted trichinæ, even though it acts during a period of 11 days.

2. A temperature of −6° C. (21.2° F.) is easily withstood by trichinæ during a period of 10 days, but they revive slowly.

3. A temperature of −9° C. (15.8° F.) is sometimes fatal, but not always. The results are not always the same; they are uncertain.

4. A temperature of −15 to −16° C. (5° to 3.2° F.) is always fatal; the trichinæ never revive.

Winn (1915) exposed some trichinous meat out of doors away from the sun in February, 1914, for 16 days, at an average mean temperature of −18.8° C. (−2° F.) with a minimum of −25° C. (−13° F.) and a maximum of −12.2° C. (10° F.). Nine guinea pigs were fed upon this meat, and none became infested.

EXPERIMENTAL WORK

DESCRIPTION OF EXPERIMENTS

The first experiment was carried out in Chicago in September, 1913. The carcass of a naturally infested trichinous rat killed on September 11 was inclosed in a tin can and kept in a refrigerator until September 16, when it was placed in a refrigerated compartment known as a "freezer" in one of the meat-packing establishments, where it remained for nearly 6 days—i. e., 5 days, 22 hours. During this time the temperature (as recorded by a thermometer not compared with a standardized thermometer), read once daily, varied from $-3°$ to $-10°$ F.[1] When removed, the rat carcass was allowed to thaw by exposure to ordinary room temperature, after which eight trichinæ were isolated by dissection. Examined in water on a warm stage, they were found to be shrunken and motionless. They were left in a moist chamber and again examined the following day, when they were found to be no longer shrunken, but exhibited no movement. Two more trichinæ, isolated from the meat the day after removal from the freezer, were also found to be inactive. A guinea pig was fed some of the meat from the rat carcass on September 25 and was found to be free from trichinæ when examined on October 25.

The failure to discover any evidence of life among the trichinæ isolated from the frozen rat carcass led to further experiments.

In experiment 2, a small piece of the diaphragm of another trichinous rat, after the carcass had been kept in an ice box for 11 days, was sealed in a vial and kept in a freezing mixture at a temperature of 4° to 10° F. for 30 minutes. No active trichinæ were found on examination after thawing. The rest of the carcass of the same rat was then inclosed in a tin can and placed in a freezer maintained at a temperature of 13° to 15° F., recorded by means of a thermometer (six readings daily), afterwards compared with a standardized thermometer (experiment 3). After nearly 2 days (45½ hours) the can was removed from the freezer. Trichinæ isolated by dissection soon after the meat had thawed and examined in water on a warm stage were found to be shrunken and motionless, but resumed their normal appearance and became active in 10 to 30 minutes.

In experiments 4, 5, and 6, pieces of diaphragm of an artifically infested rabbit were sealed in small vials and exposed to a temperature of $-6°$ F. for 10, 20, and 30 minutes, respectively; none of the trichinæ isolated by dissection from the meat after thawing showed any activity, and guinea pigs fed with the meat failed to become infested.

In experiment 7 the carcass of a naturally infested rat was kept in a tin can in a freezer at 13° to 15° F. (six readings daily; thermometer

[1] Because of the practical bearing of the experiments upon the meat-packing industry, refrigeration temperatures are given according to the Fahrenheit scale, which is the only temperature scale in common use in the United States.

compared with a standardized thermometer) for a period of nearly five days. Trichinæ isolated by dissection showed slight activity on a warm stage.

The methods employed in experiments 8 to 127 and a general discussion of these experiments are given in the following pages, but it has been found expedient in order to save space to omit from the narrative statements of the results. These are later set forth in tabular form (Tables I, II).

In experiment 8, a leg of the rabbit referred to in experiments 4 to 6 was inclosed in a tin can and kept in a freezer at −2° F. for 43½ hours (thermometer not compared with a standardized thermometer; one reading daily). The next day after its removal from the freezer some of the meat was chopped in fine pieces and placed in the incubator (38° to 40° C.) in a beaker containing an artificial gastric juice (water; hydrochloric acid, about 0.35 per cent; and pepsin—exact quantity of pepsin used not recorded). Unfrozen meat from the same rabbit was similarly treated, using a portion of the same lot of digesting fluid. After incubating overnight, the sediment in the beakers was washed with several changes of water by decanting and settling. Trichinæ from the two lots of digested meat were then examined in water on a warm stage and the number of active and inactive individuals recorded. A guinea pig was fed some of the meat after it had thawed, and another guinea pig was fed some unfrozen meat from the same rabbit as a control, both being killed and examined for trichinæ after the lapse of a month.

Substantially the same methods of examination and feeding of test animals, with control examinations and feedings, were employed in experiments 9 to 22b. Meat from trichinous rats and rabbits was inclosed in tin cans, placed in freezers, which were maintained at various temperatures, and kept there for various periods. Portions of the meat were digested in artificial gastric juice and washed and examined as in experiment 8. Guinea pigs were used as test animals in experiments 9 to 15, white rats in experiments 16 to 22b.

In experiments 23 to 34 the carcass of a hog which had been artificially infested with trichinæ by feeding trichinous meat from various sources at intervals during a period of four months was hung in a freezer, the temperature of which was recorded by means of a thermometer (six readings daily) which had been compared with a standardized thermometer. The dressed carcass weighed about 150 pounds. The head was removed and kept unfrozen in a cooler to provide material for control examinations and feedings. From time to time portions of the carcass were removed for examination and test feedings. The same methods of examination were followed as in experiment 8. Test animals, usually white or hooded rats, were fed, and one lot of rats was fed unfrozen meat from the same carcass as a control.

In experiments 35 to 48 the carcass of another hog artificially infested as in the case of the hog used in experiments 23 to 34, weighing about 125 pounds dressed, was split in halves, which were hung in two freezers kept at different temperatures. The same procedure as to examination and feeding of test animals was followed as in experiments 23 to 34.

In experiments 49 and 50 digested meat from a trichinous rabbit, after washing and sedimenting with water, was inclosed in small vials, frozen by immersion in a freezing mixture, and the trichinæ, after thawing, examined on a warm stage.

In experiments 51 to 55, a hog artificially infested as in experiments 23 to 48 was slaughtered, and meat from the carcass inclosed in five 1-pound cans which were placed in the center of five barrels 28 inches high by 17 inches in diameter at the ends and 20 inches in diameter at the middle, each containing about 250 pounds of pork trimmings. The head of the carcass was kept unfrozen in a cooler to provide material for control examinations and feedings. The barrels were placed in a freezer the temperature of which was recorded six times daily by means of a thermometer which had been compared with a standardized thermometer. The barrels were removed from the freezer after 7, 8, 9, 10, and 11 days, respectively, and allowed to thaw sufficiently to permit the removal of the cans of trichinous meat. Examinations of the meat were made as in experiment 8. White or hooded rats in lots of five or six were fed some of the meat on several successive days, a separate lot being fed from each can.

In connection with experiments 51 to 55, it may be noted that in another experiment it was found that the interior temperature (determined by an electrical thermometer) of a barrel containing 250 pounds of pork trimmings did not fall to the temperature of the freezer (5° to 7° F.) from an initial temperature of 32° until the barrel had been in the freezer for eight days.

In experiments 56 to 64 the carcass of the hog from which meat was taken for use in experiments 51 to 55 was hung in the same freezer, and portions were removed from time to time for examination and feeding of test animals, following the same procedure as in those experiments.

In experiments 1 to 64, specially reared white or hooded rats were used as test animals whenever possible, but in some cases it was necessary, on account of the lack of a sufficient supply, to utilize rats whose previous history was not fully known; and in other cases the use of guinea pigs was necessary. In the remaining experiments, 65 to 127, only white or hooded rats were used which had been specially reared for the purpose on food from which there was no possibility of acquiring an accidental infection with trichinæ.

The meat from six hogs was used in experiments 65 and 65a. Four of these were artificially infested hogs which had been fed with trichinous pork several months before they were slaughtered, in October, 1914.

The two others slaughtered about the same time were naturally infested, having been found trichinous on microscopic examination. A shoulder was taken from each carcass and kept unfrozen in a cooler to provide material for control examinations and feedings.

In experiment 65, trimmings were taken from each of the six carcasses and a quantity weighing 106 pounds was inclosed in a wooden box measuring 28 by 19 by 6½ inches. The box was placed in a freezer, where it remained for 19 days, the temperature of the freezer being recorded three times daily by a thermometer which was afterwards compared with a standardized thermometer. After removal from the freezer the box was allowed to thaw for 2 days. A portion of the meat was then taken from the middle, passed twice through a meat chopper, and digested and examined as in experiment 8, a control examination being made of a mixture of unfrozen meat from the same carcasses similarly prepared and digested. A definite formula was followed in the preparation of the digesting fluid, which was mixed in the following proportions: Water, 1,000 c. c.; hydrochloric acid (sp. gr. 1.19), 10 c. c.; scale pepsin (U. S. P.), 2.5 gm. Five rats were fed some of the ground meat, 50 gm. of which were placed in their cage on each of three days, a total of 150 gm., an average of 30 gm. per rat. As controls five rats were fed once an average of 10 gm. of unfrozen meat from one of the hog carcasses, another lot of five, 10 gm. each from another carcass, and so on—i. e., 30 rats in all, 5 for each hog.

In experiment 65a, some of the same lot of frozen trimmings were used and were examined and fed to five rats, following the same methods as in experiment 65. In this case the trimmings had been made into sausage meat after thawing, a curing mixture having been mixed with the meat, containing salt equivalent to 3⅓ per cent of the weight of the meat. After the addition of the curing mixture and until it was prepared for artificial digestion and feeding of test animals, the meat remained for two days in a cooler at a temperature of 36° to 37° F. Analysis showed that the meat contained 3.12 per cent of salt. In preparing it for examination and feeding tests, the meat, immediately after it was ground up, was washed in water to remove the salt.

In experiment 66, 8 pounds of meat from a naturally infested hog were inclosed in a box 15¾ by 9 by 3 inches and placed in a freezer the temperature of which was recorded three times daily by means of a thermometer which was afterwards compared with a standardized thermometer. After 19 days the box was removed and some of the meat was examined and fed to test animals, following the methods used in experiment 65. As controls, five rats were fed 50 gm. of unfrozen meat from the same carcass, an average of 10 gm. per rat.

In experiments 67 to 71, meat was taken from the same carcasses as that used in experiment 65. Mixed meat from the six hogs was placed in five half-pound tin cans. Each can contained an approximately

equal quantity of meat from each hog. Two of the cans were placed in freezers, one maintained at −9° to 0° F. (three readings daily; thermometer not compared with a standardized thermometer), the other maintained at 10° to 12° (three readings daily; thermometer compared with a standardized thermometer). When removed from the freezers, the cans were thawed at room temperature, the thawing of the meat from the can taken from the second freezer (10° to 12°) being hastened by pulling the pieces of meat apart (experiment 71). The examination and the feeding of test animals were carried out in the same manner as in experiment 65. The three other cans were placed in the center of boxes 28 by 19 by 6½ inches, each containing about 100 pounds of pork trimmings. These boxes were placed in the same two freezers as the loose cans, two in the freezer maintained at the lower temperature (experiments 67, 68), the third box in the other freezer (experiment 70). When removed from the freezer, the boxes were allowed to thaw for two days. The cans were then removed and the meat examined and fed to rats, following the methods used in experiment 65.

In experiments 72 to 76 meat was taken from an artificially infested hog which had been fed trichinous meat several months prior to its slaughter in November, 1914, and this meat was inclosed in five half-pound tin cans. A ham from the carcass was kept unfrozen, at first in a cooler and afterwards in an ice box, to provide material for control examinations and feedings. Two of the cans were placed in a freezer maintained at a temperature of −9° to 2° F. (three readings daily; thermometer not compared with a standardized thermometer), two in a freezer maintained at a temperature of 10° to 13° (three readings daily; thermometer compared with a standardized thermometer), and the fifth in the center of a box 28 by 19 by 6½ inches, containing about 100 pounds of pork trimmings, this box being placed in one of the freezers (−9° to 2°) just mentioned.

The meat in the loose cans was allowed to thaw rapidly when removed from the freezers; that in the box required two days to thaw so that the can could be readily removed. The same methods of examination were followed as in experiment 65, except that some of the examinations were made in a 0.6 per cent salt (sodium chlorid) solution following digestion of the meat, the digested meat in those cases being washed with a 0.6 per cent salt solution instead of water. The use of a 0.6 per cent salt solution was adopted when it was discovered that trichinæ digested out of meat commonly become inactive if kept from a half an hour to several hours in water at a temperature of 32° to 40° C. This does not occur in cold water nor in warm salt solution. In the earlier experiments the use of plain water probably led to no misleading results, however, as every examination was controlled by an examination of unfrozen meat similarly treated. The same methods with reference to the feeding of test animals were followed in experiments 72 to 76 as

in No. 65. Four rats as controls were fed a total of 20 gm. of meat on July 8, 1915, from the ham which had been kept unfrozen since the slaughter of the hog—nearly eight months. No infections resulted. The trichinæ had evidently died. Examination on August 25 of some of the meat after artificial digestion showed only a few trichinæ. These were dead and disintegrated. There is little doubt, however, that if control animals had been fed early enough, they would have become infested, since trichinæ from the unfrozen meat examined after artificial digestion as late as three weeks after slaughter of the hog were quite lively and appeared altogether normal.

In experiments 77 to 87, meat from five trichinous hogs was used. Three 1-pound cans (5½ by 2¾ inches) were filled with meat from the first hog. One of the cans was placed in the center of a box 28 by 19 by 6½ inches, containing about 100 pounds of pork trimmings, and another in the center of a barrel of pork trimmings weighing 383 pounds net (dimensions of the barrel not recorded). Two cans were filled with meat from the second hog and two each in the case of the third, fourth, and fifth hogs, and one can of meat from each hog was placed in the center of a box of trimmings, as was done with one of the cans of meat from the first hog. A shoulder from each hog was kept unfrozen to provide material for control examinations. These shoulders were kept in a cooler or an ice box, except during the time when they were in transit between Chicago and the Washington laboratory.

The five boxes and the barrel were placed in a refrigerated compartment or freezer, maintained at a temperature of $-2°$ to $5°$ F. The five loose cans were placed in a freezer maintained at 12° to 16°. The boxes were kept in the freezer for 15 days, the barrel for 23 days, and the loose cans for 17 days. During the time the meat was in the freezers the temperature was recorded three times daily, using a thermometer which was afterwards compared with a standardized thermometer, and found to be substantially correct. The temperature of the freezer in which the boxes and the barrel were kept varied from $-2°$ to $5°$ during the time the box and barrel containing meat from the first hog were in it. During the time the four other boxes were in this freezer the temperature varied from $-2°$ to 2°. The temperature of the freezer in which the five loose cans were kept varied between 12° and 16° during the time the can of meat from the first hog was in it, and between 13° and 15° during the time the four other cans were in it.

When the boxes were removed from the freezers after 15 days' exposure to cold, they were allowed to thaw slowly until the cans could be removed, which required two days (three days in one case, experiment 77). The thawing of the barrel required five days. After removal the cans were forwarded by mail from Chicago to Washington, where they were kept after arrival in an ice box or in a cooler (temperature, above 32° F.) until they could be examined. The time elapsing between removal from the

freezer and the placing of the meat in artificial gastric juice in preparation for examination varied between 6 and 12 days.

In preparing the meat for examination and feeding tests, the contents of the can were passed twice through a meat chopper, thoroughly mixing the ground meat together. Fifty gm. of ground meat from each can were placed in a beaker containing 600 c. c. of a freshly prepared artificial gastric juice made by the following formula: Water 1,000 c. c., hydrochloric acid (sp. gr. 1.19) 10 c. c., scale pepsin (U. S. P.) 2.5 gm. (experiments 77, 78); or the same formula modified by the addition of 6 gm. of sodium chlorid (experiments 79 to 87). The contents of the beaker were then stirred and carefully warmed to 40° C. and the beaker placed in an incubator (37° to 40° C.) for 18 to 24 hours. After removal from the incubator the supernatant fluid was decanted off, salt solution (0.6 per cent) added, the contents of the beaker stirred, allowed to settle, again decanted, more salt solution added, and so forth, until the supernatant fluid remained clear and transparent. As a control upon a possibly injurious effect of the digestant on the trichinæ, 50 gm. of ground unfrozen meat from the same carcasses as the frozen meat to be examined were placed in 600 c. c. of the same lot of digestant prepared for digesting the meat which had been frozen, put into the incubator, and removed at the same time as the other, washed in the same manner, and handled in all respects exactly the same as the meat which had been frozen. The sediment which remained in the beakers after washing and decanting was examined in salt solution (0.6 per cent) on a warm stage under the microscope.

In the tests on animals five white or hooded rats, reared from birth on food from which there was no possibility of acquiring an accidental infection with trichinæ, were used for testing each lot of meat. The five rats were kept together in a cage and 50 gm. of the ground meat were placed in the cage each day for three days, a total of 150 gm. of meat, or an average of 30 gm. per rat. The cage was watched to see that the meat was all eaten. It was usually eaten promptly. The rats which died within the first two weeks were examined for the presence of trichinæ in the intestine as well as in the muscles. In the case of those which died later only the diaphragm was examined. A month or more after feeding, the surviving rats were killed, and their diaphragms were examined. Through an oversight no control animals were fed with unfrozen meat from the five hogs from which the meat was obtained for use in this set of experiments (77 to 87). In view of the undoubted viability of the trichinæ in these hogs, however, as determined by the fact that the trichinæ obtained from digested unfrozen meat were practically all active, very lively, and quite normal in all respects, this omission is not of great importance.

In the next series of experiments (88 to 90), meat was taken from the shoulders of seven naturally infested hogs slaughtered during December,

1914, and was inclosed on January 17, 1915, in three 1-pound cans (5½ by 2¾ inches), each can containing meat from all seven hogs. The shoulders after slaughter of the hogs were kept in a cooler at a temperature a few degrees above 32° F., except during the time when they were in transit between Chicago and Washington. Five of the seven hogs were the same as those from which the meat for experiments 77 to 87 was taken. On January 18 the three cans were placed in three freezers in New York City where they remained until February 1, a period of 14 days or, to be exact, 13 days, 23 hours. The temperature of the freezers as determined by thermometers compared with a standard thermometer during this period was 4° to 7°, 8° to 11°, and 14° to 16° F., respectively (four readings daily). After removal from the freezers the cans were allowed to thaw at ordinary temperatures and were received for examination at the Washington laboratory on February 4.

The same routine as to the examination and feeding of experimental animals was followed as in the preceding experiments (77 to 87) except that the digesting fluid used contained only 5 gm. of sodium chlorid to each 1,000 c. c. of water, instead of 6 gm. In this case, as in the preceding set of experiments, no control animals were fed, but it happened that the test animals fed with the meat exposed to the temperature of 14° to 16° F. became infested, so that they served as a control upon those fed with meat exposed to the lower temperatures.

In the series of experiments numbered 91 to 126, the meat used was taken from six hogs slaughtered in Chicago prior to March 2, 1915, and found to be trichinous on microscopic examination. A shoulder from each of these hogs was sent in the fresh condition to Washington where it was retained in a cooler slightly above 32° F. to provide material for control examinations and feedings. The meat for the freezing experiments was inclosed in thirty-six 1-pound tin cans (5½ by 2¾ inches), some from each of the 6 hogs being placed in each can, so that each can contained a mixture of approximately equal portions of meat from all the hogs. On March 2, twelve of the cans were placed in a freezer maintained at a temperature of about 5° (5° to 6.5°), 12 in a freezer maintained at a temperature of about 10° (9° to 13°), and 12 in a freezer maintained at a temperature of about 15° (13.5 to 15°). After 10 days—on March 12—a can was removed from each of the 3 freezers and sent by mail to the Washington laboratory. The next day 3 more cans were removed as before, and so forth, the last cans being removed on March 25, after 23 days' exposure to cold. None was removed March 14 or 21, or 12 and 19 days, respectively, after they were placed in the freezers. The thermometers in these freezers, which were afterwards compared with a standardized thermometer, were read three times daily.

The same routine examination was followed as in experiments 77 to 90, described above, the formula of the digestant fluid being that used

in experiments 78 to 90—i. e., water, 1,000 c. c.; hydrochloric acid (sp. gr. 1.19), 10 c. c.; scale pepsin (U. S. P.), 2.5 gm.; sodium chlorid, 5 gm. A mixture of unfrozen meat from the six hogs was used in control examinations. As in the preceding experiments, five rats were fed meat from each can, following the same routine. Control animals were fed on June 15 with unfrozen meat from the six hogs which had been kept several months (since March) in a cooler. Meat from each hog was fed to two rats, 20 gm. being given to each two rats, an average of 10 gm. per rat.

In experiment 127, some meat from an artificially infested hog (the same hog from which meat was obtained in experiments 72 to 76) was inclosed in a half-pound tin can, which was placed in the center of a box 28 by 19 by 6½ inches containing about 100 pounds of pork trimmings. The box was placed in a freezer in Chicago, where it remained for 57 days, during which time the temperature as recorded by a thermometer afterwards compared with a standardized thermometer varied between 10° and 13° F. (three readings daily). After removal from the freezer the box was allowed to thaw for two days. The can was then removed and sent to the Washington laboratory. The same routine as to the examination and feeding of test animals was followed as in experiments 91 to 126.

There were no satisfactory control test animals in experiment 127, as the rats fed as controls in experiments 72 to 76, which would have served as controls in this experiment, were not fed until nearly eight months had elapsed since the slaughter of the hog from which the meat was obtained. No infestation resulted in these animals; the trichinæ were evidently all dead. Examination of some of the meat about six weeks later showed that the trichinæ were dead and disintegrated. The trichinæ, however, that were examined after artificial digestion of unfrozen meat from this hog as late as three weeks after slaughter appeared perfectly normal and were quite lively, and there is little doubt that control animals would have been infested if they had been fed early enough.

See Tables I and II for the results of these experiments.

TABLE I.—*Results of examinations and feeding tests in refrigeration experiments with larvæ of Trichinella spiralis*

Experiment No.	Source of meat.	Quantity of meat frozen.	Temperature of freezer.	Number of days.	Examination of trichinæ.				Tests on animals.				Remarks. (Letters in parentheses refer to lettered columns of table.)
					From frozen meat.		From unfrozen meat (controls).		Fed frozen meat.		Fed unfrozen meat (controls).		
					Number examined.	Percentage active.	Number examined.	Percentage active.	Positive.	Negative.	Positive.	Negative.	
	(a)	(b)	(c)	(d)	(e)	(f)[a]	(g)	(h)[a]	(i)	(k)	(l)	(m)	
			°F.										
1	1 rat	Carcass	−3 to −10	6	10	0	.....	.....	0	1	.....	.....	(k) Guinea pig.
2	do	Vial	4 to 10	(b)	10	0	.....	.....	.....	.....	.....	.....	
3	do	Carcass	13 to 15	2	12	100	.....	.....	0	1	1	0	(k) Guinea pig; (l, m) same as No. 8.
4	1 rabbit	Vial	−6	(c)	4	0	.....	.....	0	1	1	0	Do.
5	do	do	−6	(d)	2	0	.....	.....	0	1	1	0	Do.
6	do	do	−6	(b)	.....	.....	.....	.....	.....	.....	.....	.....	
7	1 rat	Carcass	13 to 15	5	2	100	.....	.....	.....	.....	.....	.....	(k, l) Guinea pigs; (l) slightly infested.
8	1 rabbit	Leg	−2 to −2	2	184	24	41	95	0	1	1	0	(f) Mostly sluggish; (k) guinea pig; (l, m) same as No. 8.
9	do	do	−2 to +3	3	209	2	.....	.....	0	1	1	0	(k) Guinea pig; (l, m) same as No. 8.
10	do	Part of carcass.	−2 to +3	5	606	0	104	100	0	1	1	0	
11	do	do	11 to 15	6	80	81	104	100	1	0	1	0	(f) Nearly all very lively; (g, h) same as No. 10; (i) guinea pig slightly infested; (l, m) same as No. 8.
12	do	do	−2 to +3	6	275	1−	(?)	100	0	1	1	0	(k) Guinea pig; (l, m) same as No. 8.
13	do	do	−1 to −2	5	433	0	37	100	0	1	1	0	Do.
14	do	do	−2 to +3	8	209	0	37	100	0	1	1	0	(g, h) Same as No. 13; (k) guinea pig; (l, m) same as No. 8.
15	do	do	13 to 15	12	64	0	26	100	0	1	1	0	(k) Guinea pig; (l, m) same as No. 8.
16	do	do	4 to 14	5	25	0	152	14	0	1	1	0	(g) In water 4 days after digestion.
17	do	do	12 to 14	5	100	7	152	14	0	1	1	0	(e) In water 4 days after digestion; (f) 4 out of 7 very sluggish; (g, h, l, m) same as No. 16.
18	do	do	14 to 22	5	42	5	152	14	0	1	1	0	(e) In water 3 days after digestion; (f) very sluggish; (g, h, l, m) same as No. 16.
19	1 rat	do	4 to 14	5	70	0	20	100	0	1	1	0	(g, h, l, m) Same as No. 19.
20	do	do	12 to 14	5	73	14	20	100	0	2	1	0	(l, m) Same as No. 16.
21	1 rabbit	do	4 to 14	6	.....	.....	.....	.....	0	2	1	0	Do.
21a	do	do	12 to 14	6	.....	.....	.....	.....	.....	.....	.....	.....	

[a] Percentages in columns (f) and (h) are expressed in the nearest whole numbers, except that percentages over 99.5 are expressed as 99 + and less than 0.5 as 1−.

[b] 30 minutes. [c] 10 minutes. [d] 20 minutes.

TABLE I.—*Results of examinations and feeding tests in refrigeration experiments with larvæ of Trichinella spiralis*—Continued

Experiment No.	Source of meat.	Quantity of meat frozen.	Temperature of freezer.	Number of days.	Examination of trichinæ.				Tests on animals.				Remarks. (Letters in parentheses refer to lettered columns of table.)
					From frozen meat.		From unfrozen meat (controls).		Fed frozen meat.		Fed unfrozen meat (controls).		
					Number examined.	Percentage active.	Number examined.	Percentage active.	Positive.	Negative.	Positive.	Negative.	
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(k)	(l)	(m)	
			°F.										
22	1 rat	Part of carcass.	14 to 22	10	58	31	168	100	0	1	1	0	(f) Very sluggish; (l, m) same as No. 19.
22a	1 rabbit	do	14 to 22	10					0	2	1	0	(l, m) Same as No. 16.
22b	do	do	12 to 14	10					0	2	1	0	Do.
23	1 hog	Carcass	14.5 to 15.5	7	476	98	208	97	6	0	2	1	(i) Four heavily, 2 moderately infested; (l, m) same as No. 34.
24	do	do	14.5 to 15.5	9	216	99+	120	100	6	0	2	1	(i) Four heavily infested; 1 degree of infestation not recorded; 1 guinea pig slightly infested; (l, m) same as No. 34.
25	do	do	14.5 to 16.5	10	180	59	182	98	0	4	2	1	(f) Sixty-three out of 107 sluggish; (k) 6 fed, 1 died after first feeding, another lost from cage; meat (loin) fed very lightly infested; (l, m) same as No. 34.
26	do	do	14.5 to 16.5	11	273	76	63	87	2	4	2	1	(f) One hundred and forty-five out of 208 sluggish; (i) slightly infested; (l, m) same as No. 34.
27	do	do	14.5 to 16.5	12	107	95	61	99	0	6	2	1	(f) Fifty-seven out of 102 sluggish; (l, m) same as No. 34.
28	do	do	14.5 to 16.5	13	59	42	61	99	0	6	2	1	(f) Twenty out of 25 sluggish; (g, h) same as No. 27; (l, m) same as No. 34.
29	do	do	14.5 to 16.5	14					6	0	2	1	(i) Three moderately, 2 slightly, 1 heavily infested; (l, m) same as No. 34.
30	do	do	14.5 to 16.5	16	102	74	99	99	2	1	2	1	(i) Slightly infested; (l, m) same as No. 34.
31	do	do	14.5 to 16.5	18	122	99	99	99	2	1	2	1	(g, h) Same as No. 30; (i) slightly infested; (l, m) same as No. 34.
32	do	do	14.5 to 16.5	21	135	18	123	80	0	3	2	1	(e) Meat digested 2 days; (g) meat from another carcass digested 2 days; (l, m) same as No. 34.
33	do	do	14.5 to 16.5	22	110	84	200	100	0	3	2	1	(f) Fifty-three out of 92 sluggish; (g) meat from another carcass; (k) guinea pigs; (l, m) same as No. 34.
34	do	do	14.5 to 16.5	24					0	3	2	1	(i) Heavily infested.
35	do	Half of carcass.	8.5 to 9.5	5	267	16	80	100	1	2	2	1	(i, k) Guinea pigs; (i) lightly infested; (l, m) same as No. 42.
36	do	do	8.5 to 9.5	6	452	2	75	100	0	3	2	1	(f) Sluggish; (k) guinea pigs; (l, m) same as No. 42.

37	...do.....	.....do.....	8.5	to	10.5	7	392	4	100	100	0	3	2	1	(f) Very sluggish; (l, m) same as in No. 41.
38	...do.....	.....do.....	8.5	to	10.5	8	298	0	133	80	.....	.....	.....	.....	(e, g) Meat digested 2 days.
39	...do.....	.....do.....	8.5	to	10.5	9	731	1—	200	100	0	3	2	0	(f) Very sluggish; (l, m) same as No. 41.
40	...do.....	.....do.....	8.5	to	10.5	10	701	1—	200	100	0	3	2	0	(f) One out of 3 sluggish, 2 very sluggish; (g, h) same as No. 39; (l, m) same as No. 41.
41	...do.....	.....do.....	8.5	to	10.5	11	.....	.....	.....	.....	0	3	2	0	(g, h) Same as No. 35; (i) 2 moderately, 1 slightly infested; (l, m) same as No. 41.
42	...do.....	.....do.....	5	to	7	5	221	14	80	100	3	0	2	0	(g, h) Same as No. 36; (k) guinea pigs; (l, m) same as No. 41.
43	...do.....	.....do.....	5	to	8	6	159	0	75	100	0	3	2	0	(f) Very sluggish; (g, h) same as No. 37; (l, m) same as No. 41.
44	...do.....	.....do.....	5	to	8	7	449	1—	100	100	0	3	2	0	(e) Meat digested 2 days; (g, h) same as No. 38.
45	...do.....	.....do.....	5	to	8	8	326	0	123	80	.....	.....	.....	.....	(f) Very sluggish; (g, h) same as No. 39; (l, m) same as No. 41.
46	...do.....	.....do.....	5	to	8	9	603	1—	200	100	0	3	2	0	(g, h) Same as No. 39; (l, m) same as No. 41.
47	...do.....	.....do.....	5	to	8	10	517	0	200	100	0	3	2	0	(i) Old infection, encysted trichinæ in diaphragm; rat died 7 days after first feeding; (l, m) same as No. 41.
48	...do.....	.....do.....	5	to	8	11	.....	.....	.....	.....	1	2	2	0	(b) Sediment of digested meat in small vial.
49	1 rabbit	Vial.....	7	to	10	(a)	354	0	37	100	.....	.....	.....	.....	(b) Sediment of digested meat in small vial; (f) sluggish; (g, h) same as No. 49.
50	...do.....	.....do.....	10	to	18	(b)	230	3	37	100	.....	.....	.....	.....	
51	1 hog....	250 pounds..	10	to	12	7	12	100	100	100	5	0	.....	.....	(i) Heavily infested.
52	...do.....	.....do.....	9	to	12	8	.....	.....	.....	.....	6	0	.....	.....	Do.
53	...do.....	.....do.....	9	to	12	9	100	95	29	93	6	0	.....	.....	Do.
54	...do.....	.....do.....	9	to	12	10	21	90	29	93	5	1	.....	.....	(i) Heavily infested; (g, h) same as No. 53; (k) died 3 days after first feeding.
55	...do.....	.....do.....	9	to	12	11	62	71	21	100	6	0	.....	.....	(i) Heavily infested.
56	...do.....	Carcass.....	10	to	11	6	400	46	500	99	6	0	.....	.....	(i) Three heavily infested, 3 died early, few trichinæ found.
57	...do.....	.....do.....	10	to	12	7	.....	.....	.....	.....	5	0	.....	.....	(i) Heavily infested, one very heavily infested.
58	...do.....	.....do.....	9	to	12	8	105	71	100	100	4	0	.....	.....	(g, h) Same as No. 51; (i) 5 rats fed, 1 died, eaten by others, 1 slightly infested, 2 heavily infested, 1 number of trichinæ not recorded.
59	...do.....	.....do.....	9	to	12	9	.....	.....	.....	.....	5	1	.....	.....	(i) Slightly infested; (k) died 4 days after first feeding.
60	...do.....	.....do.....	9	to	12	10	.....	.....	.....	.....	6	0	.....	.....	(i) Heavily infested.
61	...do.....	.....do.....	9	to	12	11	.....	.....	.....	.....	6	0	.....	.....	(i) Three heavily, 3 moderately infested.
62	...do.....	.....do.....	9	to	12	12	48	83	29	93	5	1	.....	.....	(g, h) Same as No. 53; (i) 3 heavily, 1 slightly infested, 1 number of trichinæ not recorded; (k) died 1 day after first feeding.
63	...do.....	.....do.....	9	to	12	13	9	11	21	100	2	4	.....	.....	(g, h) Same as No. 55; (i) 1 trichina in each; (k) 1 died 4 days after first feeding.
64	...do.....	.....do.....	9	to	12	14	.....	.....	.....	.....	0	6	.....	.....	(i) Very slightly infested; (l) all but 1 heavily infested.
65	6 hogs..	100 pounds..	10	to	13	19	523	31	127	98	4	1	28	1	(b) Meat after thawing cured with salt 2 days before
65a	...do.....	.....do.....	10	to	13	19	578	3	195	98	2	2	28	1	examination and feeding; (i) very slightly infested, 4 trichinæ in each; (i, k) 5 rats fed, 1 died early, not examined; (l, m) same as No. 65.
66	1 hog....	8 pounds.....	11	to	13	19	105	100	116	98	0	4	5	0	(k) Five rats fed, 1 lost from cage; (l) 2 heavily, 3 slightly infested.
67	6 hogs..	100 pounds..	— 4	to	0	5	121	100	50	100	4	0	28	1	(i) Heavily infested, 5 rats fed, 1 lost from cage; (l, m) same as No. 65.

a 25 minutes.

b 30 minutes.

TABLE I.—*Results of examinations and feeding tests in refrigeration experiments with larvæ of Trichinella spiralis*—Continued

Experiment No.	Source of meat.	Quantity of meat frozen.	Temperature of freezer.	Number of days.	Examination of trichinæ.				Tests on animals.				Remarks. (Letters in parentheses refer to lettered columns of table.)
					From frozen meat.		From unfrozen meat (controls).		Fed frozen meat.		Fed unfrozen meat (controls).		
					Number examined.	Percentage active.	Number examined.	Percentage active.	Positive.	Negative.	Positive.	Negative.	
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(k)	(l)	(m)	
			°F.										
68	6 hogs	100 pounds	− 9 to 0	10	285	1	1,118	99	1	3	28	1	(f) One out of 2 sluggish; (i) 3 trichinæ; (i, k) 5 rats fed, 1 died early, not examined; (l, m) same as No. 65.
69	...do	½ pound	− 9 to 0	10	521	0	1,118	99	0	5	28	1	(g, h) Same as No. 68; (l, m) same as No. 65.
70	...do	100 pounds	− 9 to 0	14	1,442	1	436	99+	0	4	28	1	(f) Very sluggish; (k) 5 rats fed, 1 died early, not examined; (l, m) same as No. 65.
71	...do	½ pound	10 to 12	17	348	54	155	99	1	2	28	1	(f) Less active than normal, 79 out of 188 very sluggish; (i, k) 5 rats fed, 2 died, eaten by others; (i) 4 trichinæ; (l, m) same as No. 65.
72	1 hog	...do	− 9 to 0	15	83	0	100	99	0	5	0	4	(l, m) Meat not fed until nearly 8 months after slaughter.
73	...do	...do	10 to 12	15	117	76	100	99	1	4	0	4	(f) Less active than normal, 38 out of 89 very sluggish; (i) identity questionable, numerous well-encysted trichinæ 25 days after first feeding; (g, h, l, m) same as No. 72.
74	...do	100 pounds	− 9 to + 2	18	600	0	198	100	0	4	0	4	(k) Five rats fed, 1 died, eaten by others; (l, m) same as No. 72
75	...do	½ pound	− 9 to + 2	20	386	0	198	100	0	4	0	4	(g, h) Same as No. 74; (l, m) same as No. 72.
76	...do	...do	10 to 13	20	669	48	198	100	0	4	0	4	(f) Mostly less active than normal, 219 out of 319 sluggish; (g, h) same as No. 74; (l, m) same as No. 72.
77	...do	100 pounds	− 2 to + 5	15	153	0	193	99+	0	5	.....	.....	
78	...do	1 pound	12 to 16	17	139	50	193	99+	0	5	.....	.....	(f) Mostly quite lively; (g, h) same as No. 77
79	...do	380 pounds	− 2 to + 5	23	90	0	138	100	0	5	.....	.....	(g, h) Same as No. 79.
80	...do	100 pounds	− 2 to + 2	15	142	0	138	100	0	5	.....	.....	(f) Mostly quite lively; (g, h) same as No. 79.
81	...do	1 pound	13 to 15	17	304	65	138	100	0	5	.....	.....	(g, h) Same as No. 79.
82	...do	100 pounds	− 2 to + 2	15	152	0	138	100	0	5	.....	.....	(f) Mostly sluggish; (g, h) same as No. 79.
83	...do	1 pound	11 to 15	17	223	42	138	100	0	5	.....	.....	
84	...do	100 pounds	− 2 to + 2	15	50	0	120	100	0	5	.....	.....	(f) Mostly sluggish; (g, h) same as No. 84.
85	...do	1 pound	11 to 15	18	86	38	120	100	0	4	.....	.....	(g, h) Same as No. 84; (i) died 4 days after first feeding.
86	...do	100 pounds	− 2 to + 2	15	202	0	120	100	1	4	.....	.....	1 live, 2 dead larvæ in intestine, undigested.

87	...do.....	1 pound.....	13	to	15	18	205	67	120	100	0	5	.....	.....	(f) Mostly quite lively, but paler than normal; (g, h) same as No. 84.
88	7 hogs...	.....do.....	4	to	7	14	744	1	143	100	0	5	5	0	(f) Very sluggish; (i) test rats Exp. No. 90, serve as controls for Exp. Nos. 88, 89.
89	...do.....	.....do.....	8	to	11	14	121	98	43	100	2	3	5	0	(f) Not so lively as and paler than normal; (i) slightly infested; (l, m) same as No. 88.
90	...do.....	.....do.....	14	to	16	14	42	100	43	100	5	0	.....	.....	(g, h) Same as No. 89; (i) heavily infested.
91	6 hogs...	½ pound...	5	to	6.5	10	213	0	150	100	0	5	11	1	(i) Eight heavily infested, degree of infestation not recorded in 3; (m) killed 4 days after feeding, only small portion of intestine examined.
92	...do.....	.....do.....	5	to	6.5	11	300	0	150	100	0	5	11	1	(g, h, l, m) Same as No. 91.
93	...do.....	.....do.....	5	to	6.5	13	151	0	100	100	0	5	11	1	(l, m) Same as No. 91.
94	...do.....	.....do.....	5	to	6.5	14	204	2	55	100	8	5	11	1	(f) Very sluggish; (l, m) same as No. 91.
95	...do.....	.....do.....	5	to	6.5	15	202	0	100	100	0	5	11	1	(l, m) Same as No. 91.
96	...do.....	.....do.....	5	to	6.5	16	139	0	153	99	0	5	11	1	(e, g) Digested nearly 2 days; (l, m) same as No. 91.
97	...do.....	.....do.....	5	to	6.5	17	267	0	150	100	0	5	11	1	(l, m) Same as No. 91.
98	...do.....	.....do.....	5	to	6.5	18	139	3	150	100	0	5	11	1	(f) Very sluggish; (g, h) same as No. 97; (l, m) same as No. 91.
99	...do.....	.....do.....	5	to	6.5	20	180	0	100	100	0	5	11	1	(l, m) Same as No. 91.
100	...do.....	.....do.....	5	to	6.5	21	.....	.....	.....	.....	0	5	11	1	Do.
101	...do.....	.....do.....	5	to	6.5	22	340	0	200	100	0	5	11	1	Do.
102	...do.....	.....do.....	5	to	6.5	23	105	0	(?)	100	0	5	11	1	Do.
103	...do.....	.....do.....	10.5	to	13	10	(?)	Many.	150	100	5	0	11	1	(g, h) Same as No. 91; (i) heavily infested, 1 very heavily; (l, m) same as No. 91.
104	...do.....	.....do.....	10.5	to	13	11	(?)	...do.....	150	100	5	0	11	1	(g, h) Same as No. 91; (i) 4 heavily infested, 1 slightly infested; (l, m) same as No. 91.
105	...do.....	.....do.....	10.5	to	13	13	46	33	100	100	5	0	11	1	(f) Very sluggish; (i) 1 heavily infested, others with 1, 22, 35, and 126 trichinæ; (g, h) same as No. 93; (l, m) same as No. 91.
106	...do.....	.....do.....	10.5	to	13	14	50	90	55	100	3	2	11	1	(f) Less active than normal; (i) 20, 7, and 4 trichinæ; (g, h) same as No. 94; (l, m) same as No. 91.
107	...do.....	.....do.....	10.5	to	13	15	36	78	100	100	0	5	11	1	(f) Less active than normal; (g, h) same as No. 95; (l, m) same as No. 91.
108	...do.....	.....do.....	10.5	to	13	16	152	5	153	99	0	5	11	1	(f) Very sluggish, digested nearly 2 days; (g, h) same as No. 96; (l, m) same as No. 91.
109	...do.....	.....do.....	10.5	to	13	17	26	29	150	100	0	4	11	1	(f) Sluggish; (k) 5 rats fed, 1 died, eaten by others; (g, h) same as No. 97; (l, m) same as No. 91.
110	...do.....	.....do.....	10.5	to	13	18	35	31	150	100	0	5	11	1	(f) Sluggish; (g, h) same as No. 97; (l, m) same as No. 91.
111	...do.....	.....do.....	9.5	to	13	20	67	18	100	100	0	5	11	1	(f) Less active than normal; (g, h) same as No. 99; (l, m) same as No. 91.
112	...do.....	.....do.....	9	to	13	21	.....	.....	.....	.....	0	5	11	1	(l, m) Same as No. 91.
113	...do.....	.....do.....	9	to	13	22	(?)	(?)	200	100	0	5	11	1	(f) Sluggish; (g, h) same as No. 101; (l, m) same as No. 91.
114	...do.....	.....do.....	9	to	13	23	74	34	(?)	100	0	5	11	1	(f) Sluggish; (g, h) same as No. 102; (l, m) same as No. 91.
115	...do.....	.....do.....	14.5	to	15	10	(?)	Many.	150	100	5	0	11	1	(f) Commonly less active than normal; (g, h) same as No. 91; (i) 4 heavily, 1 very heavily infested; (l, m) same as No. 91.
116	...do.....	.....do.....	13.5	to	15	11	(?)	...do.....	150	100	5	0	11	1	(f) Commonly less active than normal; (g, h, l, m) same as No. 91.

(i) Four heavily, 1 very heavily infested.

TABLE I.—*Results of examinations and feeding tests in refrigeration experiments with larvæ of Trichinella spiralis*—Continued

Experiment No.	Source of meat.	Quantity of meat frozen.	Temperature of freezer.	Number of days.	Examination of trichinæ.				Tests on animals.				Remarks. (Letters in parentheses refer to lettered columns of table.)
					From frozen meat.		From unfrozen meat (controls).		Fed frozen meat.		Fed unfrozen meat (controls.		
					Number examined.	Percentage active.	Number examined.	Percentage active.	Positive.	Negative.	Positive.	Negative.	
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(k)	(l)	(m)	
			°F.										
117	6 hogs	½ pound	13.5 to 15	13	(?)	Many.	100	100	5	0	11	1	(f) Commonly less active than normal; (g,h) same as No. 93; (l,m) same as No. 91. (i) Three heavily, 1 very heavily infested, 1 with 43 trichinæ.
118	...do	...do	13.5 to 15	14	95	99	55	100	4	1	11	1	(f) Mostly quite lively; (i) 2 heavily infested, 2 with 9 and 3 trichinæ; (g,h) same as No. 94; (l,m) same as No. 91.
119	...do	...do	13.5 to 15	15	(?)	Many.	100	100	5	0	11	1	(f) Mostly quite lively; (i) heavily infested; (g,h) same as No. 95; (l,m) same as No. 91.
120	...do	...do	13.5 to 15	16	175	26	153	99	5	0	11	1	(f) Very sluggish, digested 2 days; (i) 4 heavily infested, 1 with 4 trichinæ; (g,h) same as No. 96; (l,m) same as No. 91.
121	...do	...do	13.5 to 15	17	(?)	Many.	150	100	4	1	11	1	(f) Quite lively; (i) 2 heavily infested, 2 with 5 and 6 trichinæ; (g,h) same as No. 97; (l,m) same as No. 91.
122	...do	...do	13.5 to 15	18	(?)	...do	150	100	3	1	11	1	(f) Quite lively; (g,h) same as No. 97; (i) heavily infested, 5 rats fed, 1 died, eaten by others; (l,m) same as No. 91.
123	...do	...do	13.5 to 15	20	(?)	...do	100	100	4	1	11	1	(f) Quite lively; (g,h) same as No. 99; (i) slightly infested, 1, 7, 4, and 18 trichinæ; (l,m) same as No. 91.
124	...do	...do	13.5 to 15	21	.....	.....	.....	.....	0	5	11	1	(l,m) Same as No. 91.
125	...do	...do	13.5 to 15	22	(?)	(?)	200	100	0	5	11	1	(f) Some quite lively; (g,h) same as No. 101; (l,m) same as No. 91.
126	...do	...do	13.5 to 15	23	(?)	(?)	(?)	100	3	2	11	1	(f) Fairly lively; (g,h) same as No. 102; (i) slightly infested, 12, 27, and 1 trichinæ; (l,m) same as No. 91.
127	1 hog	100 pounds	10 to 13	57	97	6	52	98	0	5	0	4	(f) Five out of 6 very sluggish, other 1 sluggish; (l,m) same as No. 72.

TABLE II.—*Summary of results of refrigeration experiments with larvæ of Trichinella spiralis exposed to various temperatures*

Exposure to about 15° F.				Exposure to about 10° F.				Exposure to about 5° F.				Exposure to about 0° F.			
Experiment No.	Number of days.	Examination.	Feeding tests.	Experiment No.	Number of days.	Examination.	Feeding tests.	Experiment No.	Number of days.	Examination.	Feeding tests.	Experiment No.	Number of days.	Examination.	Feeding tests.
3	2	+	....	50	(a)	+	....	49	(b)	−	....	4	(c)	−	−
35	5	+	....	5	+	+	+	2	(a)	−	−	5	(d)	−	−
7	5	+	−	6	+	+	+	16	5	−	−	6	(a)	−	−
17	5	+	−	11	+	+	+	19	5	−	−	8	2	+	....
18	5	+	−	36	+	+	−	42	5	+	+	9	3	+	−
20	5	+	−	56	6	+	+	21	6	....	−	10	5	−	−
21a	6	....	−	37	7	+	−	43	6	−	−	13	5	−	−
23	7	+	+	51	7	+	+	44	7	+	−	67	5	+	+
24	9	+	+	57	7	....	+	45	8	−	....	1	6	−	−
25	10	+	−	38	8	−	....	46	9	+	−	12	6	+	−
22	10	+	−	52	8	....	+	47	10	−	−	14	8	−	−
22a	10	....	−	58	8	+	+	91	10	−	−	66	10	+	+
22b	10	....	−	39	9	+	−	48	11	−	−	69	10	−	−
115	10	+	+	53	9	+	+	92	11	−	−	70	14	+	−
26	11	+	+	59	9	....	+	93	13	−	−	72	15	−	−
116	11	+	+	40	10	+	−	88	14	+	−	77	15	−	−
15	12	−	−	54	10	+	+	94	14	+	−	80	15	−	−
27	12	+	−	60	10	....	+	95	15	−	−	82	15	−	−
28	13	+	−	103	10	+	+	96	16	−	−	84	15	−	−
117	13	+	+	41	11	....	−	97	17	−	−	85	15	−	−
29	14	....	+	55	11	+	+	98	18	+	−	74	18	−	−
90	14	+	+	61	11	....	+	99	20	−	−	75	20	−	−
118	14	+	+	104	11	+	+	100	21	....	−	79	23	−	−
119	15	+	+	62	12	+	+	101	22	−	−				
30	16	+	+	63	13	+	+	102	23	−	−				
120	16	+	+	105	13	+	+								
28	17	+	−	64	14	+	−								
81	17	+	−	89	14	+	−								
83	17	+	−	106	14	+	+								
121	17	+	+	73	15	+	−								
31	18	+	+	107	15	+	−								
85	18	+	−	108	16	+	−								
87	18	+	−	71	17	+	+								
122	18	+	+	109	17	+	−								
123	20	+	+	110	18	+	−								
32	21	+	−	65	19	+	+								
124	21	....	−	65a	19	+	−								
33	22	+	−	66	19	+	−								
125	22	+	−	76	20	+	−								
126	23	+	+	111	20	+	−								
34	24	....	−	112	21	....	−								
				113	22	+	−								
				114	23	+	−								
				127	57	+	−								

[a] 30 minutes. [b] 25 minutes. [c] 10 minutes. [d] 20 minutes.

RESULTS OF EXPERIMENTS

EFFECTS OF VARIOUS LOW TEMPERATURES UPON THE VITALITY OF TRICHINÆ

In only one instance out of 34 experiments in which trichinous meat was exposed to temperatures of about 15° F. for periods ranging from 2 to 23 days were all of the trichinæ upon examination found to be inactive (experiment 15, 12 days). In most instances, although some were found to be inactive, a large proportion were commonly found to be active, not rarely as high as 98 to 100 per cent. In one case, even after 18 days' exposure (experiment 31), over 99 per cent of the trichinæ were found active on examination, and in another case after 22 days (experiment 33) 84 per cent were active.

In 38 experiments test animals were fed meat which had been exposed to about 15° F. for periods ranging from 5 to 24 days, with positive results—i. e., resultant infection—in 17 experiments and negative results in 21.

Some of the negative results were obtained in experiments in which the meat had been kept in the freezer for only 5 and 6 days; on the other hand, positive results were obtained from feeding meat which had been in the freezer for 23 days. Heavy infections were obtained from meat exposed as long as 18 days (experiment 122), but only slight infections resulted from meat kept in the freezer for 20 days or longer (seven experiments), and then only in two instances: In experiment 123 (20 days) one rat was negative, four slightly infested, and in experiment 126 (23 days) two rats were negative, three slightly infested.

From these results it appears that trichinous meat commonly fails to produce infection after exposure to temperatures of about 15° F. for periods of 5 to 24 days, notwithstanding the fact that many trichinæ remain alive and are quite lively when thawed out after such exposure. Failure to infect is probably because, first, of a reduction in the number of live trichinæ and, second, of a reduction in the vitality of those that remain alive. It may be concluded that although a temperature of 15° F. has an injurious action upon the vitality of trichinæ, this temperature is uncertain in its effects and that meat exposed to a temperature of 15° F. for as long as 23 days is still liable to produce infection. These results correspond to those obtained by Schmidt, Ponomarer, and Savelier (1915) who concluded from their experiments that a temperature of −9° C. (+15.8° F.) is sometimes fatal to trichinæ, but not always and that the results of exposure to this temperature are variable and uncertain.

The same authors also found that a temperature of −6° (+21.2° F.) has comparatively little effect upon trichinæ exposed to it for a period of 10 days.

Trichinæ were found to be alive upon examination in 34 out of 35 experiments in which trichinous meat was exposed to temperatures of about 10° F. for periods varying between 30 minutes and 57 days, all but one of the experiments having to do with periods of 5 to 23 days. In the one case in which all of the trichinæ were found to be dead (experiment 38) the meat had been artificially digested for 2 days in preparation for examination instead of less than 24 hours as usual, which is the probable explanation why none was found alive. Although there were no striking differences in the percentages of trichinæ found alive as compared with the findings in the experiments in which meat was exposed to temperatures of about 15°, it was frequently noted that they were less lively than normal, commonly sluggish. In 20 of the experiments a record was made of the degree of activity and it was noted that in 19 of these the trichinæ were sluggish, or at least less lively than

normal, and that in the twentieth they were nearly all very lively (experiment 11, 6 days' exposure). It was quite noticeable in the examinations that the activity of the trichinæ was generally much more impaired than in the case of meat exposed to 15°.

In 41 out of the total of 43 experiments in which meat was exposed to temperatures of about 10° F., test animals were fed, the results being positive in 22 cases, negative in 19. In one of the latter (experiment 73) one out of five rats was found to be heavily infested, but there is a question as to the identity of this rat; furthermore, the trichinæ were too far advanced in development to have resulted from meat fed at the time the rats belonging to this lot were fed. In feedings with meat exposed to temperatures of about 10° for 13 days or less, heavy infestations were commonly produced, but in 17 experiments with meat exposed 14 to 23 days and in one with meat exposed 57 days the results of feeding were either negative or, if infection was produced, it was slight. In only 4 of these 18 experiments did any of the test animals become infested. In experiment 106 (14 days) three rats were slightly infested, two negative; in experiment 71 (17 days) one was very slightly infested (four trichinæ in diaphragm), two negative; in experiment 65 (19 days) four were very slightly infested, one negative; and in experiment 65a (19 days) two were very slightly infested (four trichinæ in the diaphragm of each), two negative.

Summarizing the results of the experiments with meat exposed to temperatures of about 10° F. it may be noted that trichinæ have been found to survive in meat exposed for as long as 57 days, though in that case only a small percentage, and those only sluggishly active, and that some survived in nearly all cases, their numbers and vitality, however, having been so reduced that after 14 days' exposure either no infection resulted in test animals or, if infection resulted, it was very slight. Evidently, therefore, the effects of a temperature of 10° upon the vitality of trichinæ are decidedly more pronounced than those of a temperature of 15°.

Twenty-five experiments were carried out in which trichinous meat was exposed to temperatures of about 5° F.; and in 23 of these, examinations were made of the trichinæ after thawing. In only six instances were live trichinæ found. In experiment 42 (5° to 7° for 5 days) 14 per cent of the trichinæ were found to be alive, degree of activity not recorded. The number of live trichinæ found in the five other experiments ranged from less than 1 per cent to 3 per cent, and they were all very sluggish (experiments 44, 46, 88, 94, 98), the periods of exposure to cold being 7, 9, 14, 14, and 18 days, respectively.

Test animals were fed in 23 experiments. No infections resulted except in experiment 42, just referred to. In this experiment three rats were fed and two became moderately and one slightly infested.

The results of these experiments show that temperatures of about 5° F. have a profound effect upon the vitality of trichinæ. Only a very small proportion survive an exposure of more than five days, and these are so seriously affected that infections are extremely unlikely to occur, none having resulted in any case in which test animals were fed meat exposed to temperatures of about 5° for periods ranging from 6 to 23 days (19 experiments). In view of the results of experiment 68, however, in which the temperature was −9° to 0° and the period of exposure 10 days, it may be concluded that slight infections may sometimes result from meat exposed to 5° for as long as 10 days.

The results of the experiments with temperatures of about 5° F. correspond closely to those of Schmidt, Ponomarer, and Savelier (1915). These authors, however, found that in their experiments a temperature of −15° to −16° C. (3.2° to 5° F.) was always fatal to trichinæ and noted no exceptions such as were observed by the present writer.

In experiments in which trichinous meat was exposed to temperatures of about 0° F., but ranging as low as −10° in some instances, trichinæ were rarely found to be alive. However, 100 per cent were found to be alive in one experiment (No. 67) in which meat had been exposed to a temperature of −4° to 0° for 5 days, but in 15 experiments in which the period of exposure to cold ranged from 6 to 23 days trichinæ were found alive only in three instances and less than 1 per cent in each case (experiments 12, 68, and 70).

Test animals were fed in all but 1 of the 23 experiments with temperatures of about 0° F. Infection resulted in two instances. Four rats fed in experiment 67 (−4° to 0°, for 5 days) became heavily infested, and one out of four in experiment 68 (−9° to 0°, for 10 days) showed three trichinæ in the diaphragm, the three other rats being negative. In the latter case, as in the former, live trichinæ had been found by examination of the meat; less than 1 per cent, however, as compared with 100 per cent in the former, the results of the feeding tests thus as usual being quite consistent with the results of the examinations of artificially digested meat, though it was unusual for infection to result when the examination showed such a small percentage of live trichinæ as in experiment 68. In experiment 86 (−2° to +2°, for 15 days), in which no trichinæ were found alive on examination of artificially digested meat, the result of the feeding test is considered to have been negative, although one of the five test rats, which died four days after feeding, was found to have three trichina larvæ in the intestine, two of which were dead, whereas the other one exhibited feeble movements. None of these three larvæ, however, had undergone any development, and the four other test rats were negative, so that it seems quite proper to conclude that the viability of the trichinæ had been destroyed in the meat in question.

From the foregoing it appears that the results of exposing trichinous meat to temperatures of about 0° F. are similar to those produced by temperatures of about 5°—i. e., a few trichinæ may survive exposures to such temperatures for 6 days or more, but their vitality will be so greatly reduced that there is little likelihood of their causing infection, although, on the other hand, slight infections may result from meat exposed as long as 10 days.

A good example of the relative effects of different low temperatures upon the vitality of trichinæ is supplied by experiments 91 to 126. In these experiments approximately equal quantities of trichinous pork from the same source (mixture of meat from six hogs) were exposed for 10 to 23 days in three freezers at temperatures of about 15°, 10°, and 5° F., respectively, a can of meat being removed from each of the three freezers after 10 days' exposure, another after 11 days, and so on (no cans, however, being removed on the twelfth or nineteenth day). It will be observed from the recorded results (Tables I, II) that many of the trichinæ in the meat exposed to a temperature of about 15° survived, and up to the twentieth day of exposure were mostly quite lively after thawing. Some of those from meat exposed for 22 days were observed to be quite lively, and those which survived in meat exposed for 23 days were found to be fairly lively. From the results of the feeding tests there appeared to be a considerable reduction in the vitality of the parasites after 17 days' exposure, notwithstanding the survival of a large percentage. Most of the rats fed meat exposed to about 15° for 10 to 16 days became heavily infested, but the 17-day meat failed to infect one out of five rats, and only two of the four others became heavily infested, the 18-day meat failed to infect one out of five, the 20-day meat failed to infect one, the four others becoming only slightly infested, none of the rats fed 21- and 22-day meat became infested, and the 23-day meat failed to infect two and produced only light infestations in the three others.

In the case of the meat exposed to a temperature of about 10° F. it was observed that the trichinæ which survived were relatively less numerous, as a rule, than in the case of the meat exposed to about 15°, and it was generally noted that they were less active than normal, or sluggish, sometimes very sluggish. The test rats, fed meat exposed for 10 days, all became heavily infested, all five fed 11-day meat became infested, but one was only slightly infested, all five fed 13-day meat became infested, but only one was heavily infested, three out of five fed 14-day meat became infested, but these only slightly, and none of the rats fed meat exposed to about 10° for 15 days or longer became infested. In this series, therefore, there was apparently a considerable reduction in the infectiousness of the meat beginning with that exposed for 13 days, and after 2 days more the infectiousness became nil.

Practically none of the trichinæ in the meat exposed to a temperature of about 5° F. (experiments 91 to 102) survived; although living trichinæ

were observed in meat exposed for 14 and 18 days (2 and 3 per cent, respectively), these were very sluggish. Furthermore, none of the test rats in this series became infested.

The results of the three sets of experiments just cited demonstrate quite clearly that a temperature of 10° F. is more effective in destroying the vitality of trichinæ than a temperature of 15°, and that a temperature of 5° is still more effective, illustrating the general rule established by the investigations recorded in the present paper, that within certain limits the effect upon the vitality of trichinæ becomes more pronounced as the temperature of refrigeration is lowered. It has also apparently been established that the increase in effectiveness is not uniform with the decrease in the temperature, but that somewhere in the neighborhood of 10° a critical point is reached, below which there is a sudden increase in the effectiveness of refrigeration.

Summarizing the results of the various experiments with a view to their practical application, inasmuch as very few trichinæ have been found to survive an exposure of more than 10 days to a temperature of 5° F., or lower, and as the few surviving have shown only very slight activity, and as, moreover, trichinous meat exposed to temperatures of 5° or lower has rarely produced infestation, and has never (in repeated trials) produced infestation when the period of exposure was more than 10 days, it may be concluded that meat exposed to a temperature not higher than 5° for a period of 20 days will no longer contain viable trichinæ, 10 days in this 20-day period being allowed as a margin of safety. It may be further concluded that, so far as our present knowledge goes, temperatures of 10° and higher are too uncertain in their effects upon the vitality of trichinæ to justify the use of refrigeration at such temperatures as a means of rendering trichinous meat innocuous.

CHANGES PRODUCED IN TRICHINA LARVÆ BY EXPOSURE TO LOW TEMPERATURES

Low temperatures (15° F. and lower) not only destroy the vitality of some or all of the trichinæ which are exposed to those temperatures but they produce changes in the tissues of the parasites, which are apparent under the microscope. These changes in appearance are associated with reductions in the activity of the trichinæ and with losses in their vitality.

Trichinæ from artificially digested unfrozen meat when examined under the microscope in water, or preferably in a physiological salt solution are found to be tightly coiled, becoming very lively when they are warmed to body temperature and continuing their lively movements as the temperature increases up to about 50° or 52° C. when they become sluggish and finally cease movement and die when the temperature rises a few degrees higher. The esophageal cellular body of the normal trichina has a bright yellowish brown color, and exhibits a certain granulation of the protoplasm; the nuclei of the cells are apparent as small,

clear, spherical bodies, seemingly of a vesicular nature. The gonad (ovary or testis) forms a continuous mass of cells closely pressed together, intercellular divisions and nuclei being indistinct in the living specimen. The body cavity forms a thin but distinct space between the internal organs and the parietal wall. In short, the normal living trichina larva freed from its capsule by artificial digestion presents a sharp clear-cut bright appearance which is quite characteristic but difficult to describe.

The changes shown by the trichinæ from artificially digested meat in experiments 118, 106, and 94 are typical of those produced by the exposure of trichinous meat to various low temperatures. In these instances the temperatures were 13.5° to 15°, 10.5° to 13°, and 5° to 6.5° F., respectively, and the period of exposure 14 days in each case. The meat was all of the same origin—i. e., from six hogs, mixed together, portions of about half a pound being inclosed in tin cans and placed in freezers maintained at the temperatures stated. The cans were removed at the end of 14 days and the meat allowed to thaw at ordinary temperatures. Two days after removal from the freezers the meat from each can was ground up, digested overnight in an artificial gastric juice, washed and sedimented in a 0.6 per cent salt solution and the trichinæ thus obtained subjected to examination. As usual, for the purpose of controlling the results of these processes upon the frozen meat, unfrozen meat from the same carcasses was digested, washed, and examined in exactly the same manner.

Out of 95 trichinæ from the meat which had been exposed to a temperature of 13.5° to 15° F. (No. 118), only one was inactive, this one being pale in color, and the nuclei in the cellular body having a solidified appearance. The 94 others were more or less tightly coiled when cold, and most of them were quite lively when warmed. The granulation of the protoplasm of the cellular body differed only slightly from normal, and its color was nearly normal; the nuclei showed commonly a small central point of more solid appearance than the remainder of the nucleus. The gonad either showed only slight changes from normal or the germ cells were rounded instead of being closely pressed together, this rounding of the cells occurring in only a part of or throughout the gonad. Two of the test rats in this experiment became heavily infested; one was negative; one showed 9 trichinæ in the diaphragm; and one 3 trichinæ in the diaphragm.

Fifty trichinæ were examined from the meat which had been exposed to a temperature of 10.5° to 13° F. Of these, five were inactive, pale in color, their coils expanded so that they resembled a figure 6, and the nuclei of the cellular body of the esophagus were solidified. The 45 which were active were more or less tightly coiled when cold, some of them being quite lively when warmed. The color of the cellular body was rather paler than normal, the protoplasm abnormally granular, the nuclei either not apparent or exhibiting a solidified central portion. The cells of the

gonad were rounded instead of being closely pressed together as in the normal trichina. Two out of five test rats were negative, the three others contained 4, 7, and 20 trichinæ, respectively, in the diaphragm.

In experiment 94, in which the meat had been exposed to a temperature of 5° to 6.5° F., 204 trichinæ were examined, 199 of which were inactive, and only 5 of which showed any activity when warmed, this consisting of a very slight movement on stimulation with a needle point. The coils were expanded in the form of a figure 6, or in some instances formed a very loose spiral. The esophageal cellular body was very pale in color, granulation of the protoplasm very abnormal, nuclei solidified, quite different in appearance from the normal vesicular nucleus. The cells of the gonad were rounded and more or less dissociated. Five test rats fed in this experiment all failed to become infested.

The abnormal granulation of the cellular body referred to is difficult to describe, but it gives the protoplasm a distinctly different appearance from that of the cellular body of an unfrozen trichina, dull and dead-looking as compared with the bright appearance of the latter, the visible particles being much more numerous and smaller.

Comparison of the results of these three experiments and similar experiments shows not only that microscopically visible changes occur in the minute structure of trichinæ subjected to temperatures of 15° F. and lower, but that these changes are more pronounced in trichinæ subjected to about 10° than in those subjected to about 15°, and still more pronounced in trichinæ subjected to about 5°. These changes are evidently brought about by the low temperature, but in what way is not apparent. This problem probably belongs in the field of colloid chemistry. There occurs perhaps a precipitation of the colloids in the tissues of the trichina or some change in their nature which is more or less irreversible, according as the temperature is lower or higher and the period of exposure longer or shorter. In those cases in which the trichinæ were examined very soon after thawing of the meat (experiments 1 and 3, for example) it was quite evident from the shriveled appearance of the parasites that fluid had been extracted from them during their exposure to cold. Trichinæ thus shriveled absorb moisture after thawing and soon lose their shriveled appearance, again becoming active unless the temperature was too low and the period of exposure to cold too long continued. In some respects trichinæ which have been frozen at a low temperature (5° F.) resemble those which have been dried and then moistened again. Ordinary drying, however, destroys the vitality of trichinæ immediately, and the changes produced are much more marked than those produced by freezing. It is possible that the latter might be more closely simulated if the trichinæ were very gradually dried and the drying process stopped at the proper point. As yet, however, careful experiments along this line have not been carried out.

In view of the recent discovery by plant physiologists (see Bachmann, 1914) that sugar in plant tissues acts in some manner to protect them from the injurious effects of freezing so that the same species of plant is able to withstand a lower temperature when its tissues are loaded with sugar than when they contain only small quantities of this substance, it is of interest to note that larval trichinæ contain a high percentage of glycogen.

Whatever may be the explanation of the destruction of the vitality of trichinæ and of the changes brought about by exposure to cold, the investigations thus far carried out are sufficient to prove that trichinæ when exposed to temperatures of 15° F. or lower undergo changes in their protoplasmic structure, and if the temperature is low enough and the exposure to cold continued long enough these changes become so pronounced and so well established that the vitality of all of the parasites is entirely destroyed.

VARIATIONS IN VITALITY OF TRICHINÆ

It is natural to expect that individual trichinæ would vary in resistance to the effects of cold, and this was found to be the case. Some succumb much more quickly and at higher temperatures than others. In order to avoid misleading results on this account, meat was not used in the experiments unless heavily infested so that large numbers of trichinæ might be available for study, considerable quantities were used, as a rule, for examination and for feeding tests, several test animals (four to six) being generally employed; and, commonly, mixed meat from several hogs was used so that the chances of including only feebly resistant trichinæ in an experiment may be considered to have been reduced to a minimum in most cases.

QUANTITIES OF MEAT FROZEN

As already noted, various quantities of meat ranging from a gram or two up to nearly 400 pounds in weight were frozen in the various experiments The rate of freezing and thawing, of course, varied with the quantity of meat, the change of temperature being rapid when small quantities, slow when large quantities were used. When very small quantities of meat or of fluid containing free trichinæ were frozen and thawed within a few minutes (experiments 2, 4, 5, 6, 49, 50) the trichinæ were apparently much more injuriously affected than when larger quantities of meat were subjected to similar temperatures for considerably longer periods of time. On the other hand, if the quantity of meat weighed half a pound or more, differences in the weight, and consequently in the rate of freezing and thawing, made no appreciable difference in the effect upon the vitality of the trichinæ, as is quite evident from a comparison of the various experiments recorded in the tables. In short, it may be

stated that if the temperature to which trichinous meat is exposed is sufficiently low and the length of exposure sufficiently long, the trichinæ are killed just as certainly when large quantities of meat are frozen as when small quantities (not less than half a pound) are frozen, variations in the rate of freezing and thawing dependent upon variations in the quantity of meat frozen being immaterial.

VARIATIONS IN LENGTH OF TIME AFTER REMOVAL FROM FREEZER BEFORE EXAMINING AND TESTING MEATS

In some cases examination of the trichinæ from meat which had been frozen was made on the same day the meat was removed from the freezer or freezing mixture. When the meat was digested before examination, it was in some instances placed in the digesting fluid the same day the meat was removed from the freezer, but generally one or more days up to a maximum of 12 days elapsed before the meat was digested and examined, and a corresponding period before the feeding of test animals was begun.

Nearly all of the experiments were carried out in cold weather, and the meat after thawing, except when in transit to the laboratory, was kept in coolers or ice boxes until it was placed in a digesting fluid or fed to test animals, so that decomposition changes were slight.

In the majority of instances the meat was placed in digesting fluid in preparation for examination and the feeding of rats begun within four days after removal from the freezer, but longer periods appeared to have no pronounced effect upon the results. Certainly the lapse of time did not favor the revival of the trichinæ. For example, in experiments 77, 80, 82, 84, and 86 the periods which elapsed between removal from the freezer (about 0° for 15 days) and the digestion of the meat were 12, 8, 8, 10, and 10 days, respectively; and between removal from the freezer and the first feedings of test animals, 13, 8, 8, 10, and 10 days, respectively, yet no trichinæ were found alive on examination, and none of the test animals became infested. On the other hand, it did not seem that the lapse of time following removal from the freezer had much effect in reducing the vitality of surviving trichinæ, though it is quite likely that the longer the period which elapses after trichinous meat is removed from the freezer the fewer the surviving trichinæ will be, other things being equal. In experiments 126, 81, 83, 85, 87, and 78, the periods elapsing between removal from the freezer (about 15°, 17 to 23 days) and digestion of the meat were 6, 6, 6, 7, 7, and 9 days, respectively, and between removal from the freezer and the first feedings of test animals 4, 6, 6, 1, 7, and 10 days, respectively. A high percentage of trichinæ were found to be alive in each case. In only one of the experiments in question (No. 126) did any of the test animals become infested, and this might be taken to indicate that the trichinæ had suffered somewhat

because of the longer periods elapsing since the removal of the meat from the freezer, inasmuch as in other experiments in which the period of exposure in the freezer had been about the same but in which the meat was fed more promptly positive results were obtained in the feeding tests—i. e., in experiments 31, 122, 123, and 121, the periods elapsing between removal from the freezer and the first feeding of test animals being 2, 2, 2, and 3 days, respectively. This comparison, however, is not of great value, since in experiments 15, 28, 27 (meat in freezer at about 15° F. for 12 to 13 days), and 125 (meat in freezer at about 15° for 22 days) in which the meat was fed 2, 5, 5, and 4 days, respectively, after removal from the freezer, the results of the feeding tests were negative.

Further investigation is required to determine the changes which occur in the vitality of trichinæ when frozen meat is kept for varying periods of time after thawing. From the data at present available, however, it is quite certain that if any considerable changes occur, they are in the direction of a lowering of vitality and not in the reverse direction.

In this connection it is of interest to note that in unfrozen meat kept over three months after slaughter the trichinæ had suffered no evident loss in vitality, and small quantities of the meat were sufficient to produce heavy infestations in rats (controls, experiments 91 to 126). On the other hand, in meat kept nearly eight months after slaughter the trichinæ had lost their vitality, and test rats failed to become infested (controls, experiments 72 to 76).

EFFECTS OF ARTIFICIAL DIGESTION UPON TRICHINÆ

As evident from the tabular statement of the experiments (control examinations), artificial digestion for 24 hours or less had no appreciably injurious effect upon the vitality of trichinæ. When digested for two days, however, a considerable proportion of the trichinæ are liable to be killed (experiment 32). On the other hand, if 5 or 6 gm. of salt are added to each liter of digestive fluid the vitality of the trichinæ is not so seriously affected. The trichinæ from unfrozen meat digested for two days in experiment 96 seemed as lively as usual. Trichinæ, however, from meat frozen for 16 days at about 15° F. in experiment 120 evidently suffered considerably from digestion for two days, inasmuch as a smaller proportion were active and these were less lively than trichinæ examined in experiments 121, 122, 123, from meat frozen 17, 18, and 20 days, respectively, at about 15° F. and digested less than 24 hours. Furthermore, the fact that prolonged digestion in a digestive fluid containing 0.5 per cent of sodium chlorid is injurious to trichinæ from unfrozen meat was shown by an experiment in which digestion was continued for four days. In this instance all of the trichinæ were killed.

Though it is possible that the methods of artificial digestion employed in the experiments to free trichinæ from meat for examination reduced

their vitality so that many were found to be inactive which before digestion were still alive, the results of the examinations corresponded very well with the feeding tests. In fact, the examinations not uncommonly showed some of the trichinæ to be still alive, whereas in the corresponding feeding tests with the same meat not artificially digested none of the test animals became infested. On the other hand, there was no case in the freezing experiments in which the feeding test resulted in infection and the corresponding examination failed to reveal living trichinæ unless experiment 86 be taken as an exception. In this experiment, following a negative examination of digested meat, 3 larval trichinæ were found in the intestine of one of the test rats, which died four days after the first feeding; one of these larvæ was alive and exhibited feeble movements, but none of the 3 had undergone any development; the 4 other test rats failed to become infested. Experiment 67 was nearly an exception to the rule, as only 2 live trichinæ were found among 285 examined, the feeding test resulting positively. Only one out of four test rats became infested, however, and this one had but 3 trichinæ in the diaphragm. On the whole, the method of artificial digestion appears to afford a more rigorous test of the viability of trichinæ than the feeding of experimental animals in view of the fact that trichinæ are often found to be alive in digested meat when the feeding of the undigested meat to experimental animals fails to produce infection.

As a rule, in testing meat it is preferable not to depend alone upon the results of artificial digestion or the results of feeding test animals, but to employ both methods and take the results of both into consideration.

It is quite evident from the results of the experiments that artificial digestion is a valuable method for testing the viability of trichinæ, and that when properly controlled its injurious effects upon their vitality are so slight as to be practically negligible. The following formula may be recommended as fully satisfactory:

Water	1,000 c. c.
Hydrochloric acid (sp. gr. 1.19)	10 c. c.
Scale pepsin (U. S. P.)	2.5 gm.
Sodium chlorid	5 gm.

Fifty grams of ground meat are to be stirred into 600 c. c. of the digesting fluid, warmed to 38° or 40° C., and incubated for about 18 hours at this temperature.

LONGEVITY OF TRICHINÆ AFTER ARTIFICIAL DIGESTION

Trichinæ freed from their capsules by artificial digestion have been kept alive in tap water for 15 days. In one case 73 out of 75 were active at the end of this time. When examined again, 13 days later, all were dead. Kept in a 0.6 per cent sodium-chlorid solution for 16 days, 41 out of 43 examined were alive, some of them being sluggish but most of them

moderately lively. In another lot kept in a 0.6 per cent sodium-chlorid solution for 26 days, 15 out of 24 were alive and moderately active when warmed. Examined again 24 days later, all were dead. In a lot kept in 2 per cent sodium-chlorid solution for 11 days, 37 out of 38 were alive and very active. In these instances, after digestion of the meat, the trichinæ were washed in several changes of water or in physiological salt solution by decanting and settling. They were kept at ordinary room temperature. Numerous observations were made which showed that trichinæ freed from their capsules by artificial digestion will be apparently just as lively after several days if kept in water or physiological salt solution at ordinary room temperature as they are immediately after digestion.

If tap water containing trichinæ is kept at a temperature of 38° C. most of them are killed in a short time, but trichinæ may be kept an equal length of time at this temperature in a 0.6 per cent sodium-chlorid solution without apparent injury as shown by the following: Trichinæ from artificially digested meat were separated into two lots in beakers, one containing tap water, the other a 0.6 per cent sodium-chlorid solution. The two beakers were heated to 38° C. and this temperature maintained for 2½ hours. At the end of this time 23 out of 32 trichinæ from the tap water were inactive, whereas 18 examined from the salt solution were all active. The two beakers after replacing the tap water in one with a 0.6 per cent sodium-chlorid solution were kept at room temperature until the following day and then reexamined. Out of 108 trichinæ examined in the one case (heated in tap water), 81 were found to be inactive, whereas in the other case (heated in salt solution) all but 1 out of 100 examined were active.

On another occasion some trichinæ in tap water were kept at a temperature of 32° C. for about half an hour. Most of them became inactive but resumed their activity when the water was replaced with a 0.6 per cent sodium-chlorid solution, although their color became darker than normal and vacuoles appeared in the lateral fields.

It was on account of this discovery of the injurious effects of warm tap water that in the later experiments when meat was digested artificially it was washed with salt solution instead of tap water, and that salt solution instead of tap water was used as an examination medium. The use of tap water in the earlier experiments, however, probably affected the results of the examinations little, if any, as they are evidently quite consistent with the results of the later experiments (see Tables I and II). The washing was done with cold tap water, and in examining the trichinæ they were transferred a few at a time to a warm stage, where they were kept only a few minutes, too short a period for the injurious effects of immersion in warm water to become established, as was repeatedly demonstrated in using this method upon trichinæ from unfrozen meat.

TEST ANIMALS

It will be noted from Table I that of the 54 test animals (53 rats, 1 guinea pig) fed with unfrozen meat as controls upon the animals fed with frozen meat, only 3 failed to become infested. The rats fed as controls in experiments 72 to 76 are left out of consideration, as they were not fed until nearly eight months after the slaughter of the hog from which the meat was taken. Examination of some of the meat artificially digested nine months after slaughter of the hog showed that the trichinæ were dead. One out of three rats fed as controls in experiments 23 to 34 showed no infection, the two others being heavily infested. Out of 29 rats fed as controls in experiments 65, 65a, and 67 to 71, 1 showed no infection, 27 of the remaining 28 showing heavy infections. Finally, 1 out of 12 rats fed as controls in experiments 91 to 126 showed no infection, but this one was killed four days after feeding for another purpose and as only a small portion of the intestine was examined, trichinæ may have been present and were not discovered; 8 of the remaining rats were heavily infested; in the case of the 3 others the degree of infestation was not recorded.

These results, particularly in view of the fact that the control animals as a rule received much smaller quantities of meat than those fed on meat which had been frozen, demonstrate the adequacy of the methods employed in feeding test animals. The results of the later experiments, however, beginning with No. 23 are considered more reliable, so far as the feeding tests are concerned, than those of the earlier experiments, as more animals were used and care was taken to feed larger quantities of meat. The method of feeding each lot of test rats together in a cage a certain amount of meat on several successive days, followed in most of the experiments, appeared to be quite satisfactory. Undoubtedly some of the rats in each lot ate more of the meat than others, so that some inequality in the degree of infestation of the rats would be likely, which, however, was of little importance, as the results of the feeding tests were judged upon the basis of the findings in all of the rats in each lot. The use of a number of rats for each test allowed larger quantities of meat to be tested, which gives a decided advantage over the use of a single animal. For the same reason, rats are preferable to guinea pigs, as they will eat of their own accord much larger quantities of meat than can readily be fed to guinea pigs forcibly or by mixing with lettuce, cabbage, etc. Furthermore, it is difficult to induce guinea pigs to eat chopped meat mixed with lettuce or other materials if the meat has become only slightly tainted, whereas rats usually eat meat readily even after it has become very stale or partially decomposed.

SUMMARY AND CONCLUSIONS

Prior to the investigations recorded in the present paper very little experimental work had been done upon the effects of cold upon encysted trichinæ, and the current belief was that low temperatures do not seriously affect the vitality of these parasites. This belief is shown to have been erroneous by the results of numerous experiments.

Quantities of trichinous meat varying in weight from a few grams to nearly 400 pounds were frozen and kept for periods varying from a few minutes to 57 days at various temperatures below the freezing point of water. Usually the process of refrigeration was carried out in cold-storage compartments known as freezers, but in a few cases in which the low temperature was maintained only a short time, a freezing mixture was employed. In most cases the period of refrigeration was between 5 and 20 days. The meat on removal from the freezer was generally allowed to thaw slowly at ordinary house temperatures; in a few cases, in order to study the effects of rapid thawing, the process was hastened by breaking apart the pieces of frozen meat so that they thawed completely in a few minutes. Generally the meat after thawing was treated as follows: A portion was chopped or ground into fine pieces, placed in an artificial gastric juice, and incubated at 38° to 40° C. overnight, and then washed with water or a physiological salt solution by decanting and sedimenting. The sediment containing the trichinæ isolated from their capsules was examined microscopically on a warm stage, and the number of inactive and active ones recorded, together with such other observations as appeared worthy of remark. For the purpose of controlling the effects of the process of digestion, some trichinous meat, nearly always from the same carcass as the frozen meat, which had been kept in an unfrozen condition, was digested at the same time, using some of the same lot of digesting fluid. Another portion of the frozen meat after thawing was fed to test animals, in most cases to white or hooded rats specially reared to avoid chances of accidental infection; as a rule, five rats were fed, receiving the meat on several successive days. Finally, unfrozen meat from the same carcass as that used in a given refrigeration experiment was fed to control test animals, usually in much smaller quantities than in the case of the frozen meat. In some instances no control test animals were fed. The test animals as they died, or after about a month if they lived that long, were examined for trichinæ, the intestines as well as the diaphragm being examined if they died within the first two weeks after feeding; otherwise only the diaphragm. About 30,000 trichinæ were examined from artificially digested frozen and unfrozen meat, and over 500 test animals and control animals were fed and examined.

A considerable proportion of the trichinæ in meat exposed to a temperature of about 15° F. for periods of 23 days or less survive and are

quite lively after thawing, but such meat frequently fails to infect test animals. This temperature is injurious to trichinæ, but its effects are uncertain, and meat exposed as long as 23 days has proved to be infectious. Some of the trichinæ in meat exposed to a temperature of about 10° for periods of 57 days or less generally survive, but the meat frequently fails to infect test animals. A temperature of 10° is more injurious to trichinæ than a temperature of 15°, but, like the latter, its effects are uncertain, although meat exposed to it for 14 days or longer has generally failed to produce infestation; or if infestation resulted it was slight. No infestation has been produced by trichinous meat exposed to a temperature of about 10° for 20 days or longer.

Apparently in the neighborhood of 10° F. a critical point is reached below which the effects of cold upon trichinæ become suddenly much more pronounced.

Temperatures of 5° F. or lower profoundly affect the vitality of trichinæ. Only a very small proportion survive an exposure of more than five days, and these are so seriously affected that infections are very unlikely to result. Slight infections, however, have resulted from meat exposed to a temperature of −9° to 0° for 10 days.

Trichinæ from meat exposed to temperatures below 15° F. when examined microscopically after thawing exhibit changes in the appearance of the protoplasm. A temperature of 10° produces greater changes than 15°, and the changes produced by a temperature of 5° are still more pronounced. The more conspicuous of these changes are more or less loss of color of the esophageal cell body, more or less solidification of its nuclei, abnormal granulation of its protoplasm, and more or less dissociation and rounding of the germ cells.

Trichinæ vary in their resistance to cold, and some individuals survive refrigeration longer and at lower temperatures than others.

Within certain limits the rapidity with which trichinous meat freezes or thaws has no appreciable effect upon trichinæ. Apparently the rapid freezing and thawing undergone by very small pieces of meat (a few grams in weight) adds to the injurious effects of refrigeration, but the natural variations in the rate of freezing and thawing dependent upon variations in the quantities of meat frozen between limits of half a pound and several hundred pounds do not noticeably modify the effects of refrigeration upon trichinæ.

The vitality of trichinæ which survive refrigeration does not decrease noticeably during a period of at least a week after the thawing of the meat.

The artificial digestion of trichinous meat for 24 hours at a temperature of 38° to 40° C. in a fluid consisting of water, 1,000 c. c., hydrochloric acid (sp. gr. 1.19), 10 c. c., scale pepsin (U. S. P.), 2.5 gm., and sodium chlorid, 5 gm., has no apparent effect upon the activity or structure of the trichinæ, released from their capsules by the process of digestion.

Trichinæ thus released from their capsules may remain alive and retain their normal activity for 10 days or more when kept in a 0.6 per cent sodium-chlorid solution at a temperature of about 20° C., and have been found alive and moderately active at the end of 26 days. They may likewise be kept alive for two weeks or more in tap water at a temperature of about 20° C. Trichinæ have been kept alive and very active for 11 days in a 2 per cent sodium-chlorid solution at a temperature of about 20° C. Trichinæ in tap water warmed to a temperature of 38° C. become inactive within a few hours, but may be kept in a 0.6 per cent sodium-chlorid solution at this temperature for a similar length of time without apparent injury.

In the practical application of refrigeration as a means of destroying the vitality of trichinæ, meat should be refrigerated at a temperature not higher than 5° F. for not less than 20 days, a period which allows a probable margin of safety of nearly 10 days. The employment of higher temperatures of refrigeration as a means of destroying the vitality of trichinæ is not justified in the light of our present knowledge because of the uncertainty of the effects of such temperatures. Whether temperatures higher than 5° F. may be safely employed by lengthening the period of refrigeration remains to be determined.

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RELATION BETWEEN CERTAIN BACTERIAL ACTIVITIES IN SOILS AND THEIR CROP-PRODUCING POWER

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INTRODUCTION

Soil-bacteriological investigations in the past have dealt almost exclusively with the occurrence and activities of micro-organisms in the soil, and no attempt has been made, from the standpoint of crop production, to interpret the results obtained.

A knowledge of the relation of soil bacteria to soil fertility is of considerable importance, however, if the subject is to be of any value in practical agriculture. While, therefore, much work on methods remains to be done, so much knowledge concerning bacterial action in soils has been accumulated during the last few years that it seems time now to call attention to the practical phase of the subject, to attempt at least to correlate the results secured with known facts regarding soil fertility.

The purpose of these experiments has been to study certain bacterial activities in field soils in the attempt to secure information regarding their relation to the actual crops produced. If special methods of soil treatment exert similar effects on certain bacterial activities and on crops, it may be assumed that there is a fairly definite relation between the two, and the particular bacterial activities in a soil may indicate its crop-producing power. Thus, if in laboratory tests the ammonifying power, the nitrifying power, or the azofying power of a soil is enhanced by some method of soil treatment and the crop production is also increased, the conclusion that ammonification, nitrification, or azofication and crop production are very closely related would be well warranted. Tests of such bacterial action in soils would therefore constitute a means of ascertaining their crop-producing power, and the importance of obtaining advance information along this line is evident.

Experiments covering many years of varying seasons and including tests of all varieties of treatments must, of course, be carried out before any definite conclusions can be reached. The experiments reported here were secured on three series of plots under definite systems of treatment, and it was intended in undertaking the work to carry it on for a long period of years before attempting to draw conclusions. Inasmuch, however, as the particular plots were of necessity relinquished, owing to the development of certain departments of the State College, and studies of a like nature can not be undertaken on new plots until several years of special treatment have elapsed, it has been deemed

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expedient to assemble the data obtained up to the present time and to offer them as a preliminary contribution along this line: The fact that many of the data are rather positive in nature has been an added reason for presenting them at this time. Portions of the results have been published in other connections, while others have not previously been reported, but in either case average results only are included here.

FIELD SOILS STUDIED

Three series of field plots have been used in this work, one consisting of 14 plots one-tenth of an acre in size, located on a uniform soil in the Wisconsin drift-soil area, and classed by the United States Bureau of Soils as Carrington loam.

Prior to 1907 it had been under a regular 4-year rotation and had been subjected to no special treatment of any kind. In that year the plots were differentiated according to the following plan:

Plot No.	Treatment.
601	Continuous corn.
602, 603	2-year rotation: Corn and oats.
604, 605, 606	3-year rotation: Corn, oats, and clover.
607, 608	2-year rotation: Corn and oats, clover plowed under after the oats.
609, 610	2-year rotation: Corn and oats, cowpeas plowed under after the oats.
901, 902	2-year rotation: Corn and oats, rye plowed under after the oats.
903	Continuous clover.
904	4-year rotation: Corn, oats, and clover.

The first tests of these soils were carried out in 1911, the fourth year of the special treatment. Results were secured also in 1912 and 1913, only a few data being obtained in the latter year owing to the pressure of other work, but the ammonification studies were complete. During each season only those plots under corn were examined, as the effects of previous treatment could, of course, hardly be studied on plots under different crops, and furthermore it would be evidently impossible to compare the crop yields on the various plots if the same crop were not grown. Different plots in this series were thus examined in the different years, but in each case the same treatments were included in the study.

The second series of plots consisted of 5 one-tenth-acre plots on the same soil area and on the same soil types as the previous series. In the fall of 1910 these plots were subjected to the special treatments indicated below:

Plot No.	Treatment.
1004	Check.
1005	8 tons of manure per acre.
1006	12 tons of manure per acre.
1007	16 tons of manure per acre.
1008	20 tons of manure per acre.

The study of these plots was carried out in 1912, the crop grown that year being corn.

The third series of plots was composed of 3 one-twentieth-acre plots located on the same soil type as the other series.

Special treatment on these soils consisted in the application of lime as follows:

Plot No.	Treatment.
510	Check.
509	2 tons of ground limestone per acre.
508	3 tons of ground limestone per acre.

The lime was applied to these plots just prior to the corn planting, and the tests of the soils were carried out later in the same season.

BACTERIOLOGICAL METHODS

The solution method for testing bacterial activities in soils has been studied in some detail by several investigators, and, while results of much value have been secured by its use, there are certain difficulties attendant upon it which have not yet been obviated. These difficulties have been discussed in another publication [1] and need not be entered upon here. The use of soil itself as a medium for studying bacterial activities in field soils seems at the present time the most logical method. Modified solutions such as have been suggested in recent work [2] can hardly be considered as satisfactory as soil itself in representing the physical and chemical conditions in field soils, leaving out of account entirely the bacteriological factor.

The addition of various materials to soils in laboratory tests to permit the accumulation of the particular products of bacterial action which it is desired to measure has been studied. Dried blood, cottonseed meal, and casein have proved the best for ammonification; dried blood and ammonium sulphate for nitrification; and mannite for azofication.

In this work various modifications of the soil method were employed for the reason that the tests were carried out during a period of several years through which experiments on methods were also being conducted. The results, using the different methods, are all included, however, as they all tend in the same direction, and conclusions are based on a study of the entire mass of data secured.

EXPERIMENTAL WORK

TESTS ON ROTATION PLOTS IN 1911

Four samplings were made during 1911—on June 26, July 8, September 16, and October 25—and tests made of the soils for their ammonifying, nitrifying, and azofying powers. The yield of corn was secured from the plots examined.

[1] Brown, P. E. Methods for bacteriological examination of soils. Media for quantitative determination of bacteria in soils. Iowa Agr. Exp. Sta. Research Bul. 11, p. 379-407. 1913.

[2] Löhnis, Felix, and Green, H. H. Methods in soil bacteriology. VII. Ammonification and nitrification in soil and solution. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 40, No. 19/21, p. 457-479. 1914.

Complete data obtained in this work have been given in another place,[1] and hence only summarized results are included here.

The results of the ammonification tests with dried blood and cottonseed meal are given in Tables I and II, respectively. The nitrification tests with ammonium sulphate and dried blood appear in Tables III and IV, and the azofication results are given in Table V.

TABLE I.—*Ammonification of dried blood on rotation plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
601	171. 11	220. 74	108. 76	110. 53
602	178. 07	231. 38	117. 86	116. 54
604	188. 82	243. 60	133. 43	131. 11
607	175. 22	229. 63	129. 78	124. 83
609	179. 96	238. 53	118. 53	116. 84
901	174. 75	232. 08	117. 04	114. 88

TABLE II.—*Ammonification of cottonseed meal on rotation plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
601	142. 01	163. 32	102. 13	111. 03
602	144. 54	168. 74	110. 09	122. 17
604	151. 18	177. 81	120. 18	126. 62
607	145. 49	168. 21	131. 11	123. 49
609	148. 50	171. 00	105. 78	119. 03
901	144. 07	165. 94	112. 73	113. 55

TABLE III.—*Nitrification of dried blood on rotation plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
601	12. 442	19. 883	11. 864	13. 797
602	15. 196	23. 311	14. 629	17. 453
604	20. 776	27. 087	18. 173	24. 032
607	15. 078	22. 884	16. 410	22. 211
609	18. 798	25. 226	13. 453	15. 043
901	13. 962	20. 713	12. 711	14. 014

[1] Brown, P. E. Bacteriological studies of field soils. II. The effects of continuous cropping and various rotations. Iowa Agr. Exp. Sta. Research Bul. 6, p. 211–246. 1912.

TABLE IV.—*Nitrification of ammonium sulphate on rotation plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
601	5.019	17.577	7.565	8.086
602	8.075	21.625	9.788	11.789
604	12.630	24.517	12.903	19.419
607	7.066	21.477	11.357	13.749
609	11.908	22.978	9.101	10.620
901	6.724	21.477	8.310	9.655

TABLE V.—*Azofication tests on rotation plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
601	9.50	3.93	13.52	10.32
602	17.46	15.07	19.92	17.52
604	20.64	18.25	23.12	20.72
607	14.27	17.46	20.72	18.32
609	18.25	15.87	18.32	16.72
901	14.27	11.88	16.72	15.12

The variations in the amount of moisture in the different plots at the same samplings were very small and the differences in bacterial activities which were found could not, therefore, be attributed to the different moisture conditions in the plots.

The yields obtained with corn on the various soils are given in Table VI, and comparing these with the ammonification, nitrification, and azofication results it will be noted that there is a remarkably good agreement.

TABLE VI.—*Yield of corn on rotation plots in 1911*

Plot No.	Treatment.	Yield per acre.
		Bu.
601	Continuous corn	35.5
602	2-year rotation	46.0
604	3-year rotation	50.7
607	2-year rotation; clover turned under	52.7
609	2-year rotation; cowpeas turned under	52.5
901	2-year rotation; rye turned under	43.2

The ammonification results with the dried blood and cottonseed meal did not always run exactly parallel, but the differences were slight, and in most cases the same comparisons were secured, so they need not be

considered separately. The same is true of the nitrification results with ammonium sulphate and dried blood.

Furthermore, the ammonification, nitrification, and azofication results are all in close agreement as to the relative effects on each of the various treatments; and, hence, the bacteriological results may be compared as a whole with the crop yields.

An examination of the tables reveals the fact that a greater crop yield was secured where the 2-year rotation was followed than on the continuous corn plot, and a still greater yield was secured where the 3-year rotation was followed. Exactly the same relations were found in the ammonification, nitrification, and azofication results.

Where the clover was introduced into the 2-year rotation as a green manure a greater crop yield was obtained than where it was not used. Furthermore, a slightly greater yield was obtained than on the 3-year rotation plot. The bacteriological results are not in accord with these differences; but in most cases the variations were not large, and the differences in crop yield were not great. Hence, the lack of agreement here should not be considered of great significance.

When cowpeas were used in the 2-year rotation, however, the yield was abnormally depressed. The bacterial activities were also depressed, but not to so great an extent. Evidently some unknown factor interfered here, as such a depression is hardly explainable. Where rye was turned under in the 2-year rotation the yield was less than on the regular 2-year rotation plot, and corresponding depressions were noted in the bacterial activities.

It is apparent that the ammonification, nitrification, and azofication results as a whole show a surprisingly close relation to the crop yield. Nitrification and ammonification tests frequently proceed in the same direction, and it is possible that after many confirmatory tests have been carried out it may be found that only one of these bacteriological tests of soils needs to be made. At the present time, however, the data available along this line are insufficient to warrant the interpretation of the results from one process as fitting another.

It is hardly expected, however, that azofication results will run parallel with ammonification and nitrification tests in any large number of studies. Conditions which favor the latter processes need not necessarily favor azofication.

These results as a whole, therefore, indicate that under normal soil conditions the ammonifying and nitrifying powers of soils may reflect fairly accurately their crop-producing power and show quite accurately the relative yields which will be secured. Only in special cases can similar dependence be placed on azofication results. These tentative conclusions have been further tested and are borne out by the later results.

TESTS ON ROTATION PLOTS IN 1912

The same series of plots was used in 1912 in the study of the relative effects of different rotations on bacterial activities and on crop production, but in some cases different individual plots were employed, as again only those which were cropped to corn were examined.

Ammonification tests were carried out by the dried-blood-air-dry-soil method with inoculum from fresh soil, the casein-fresh-soil method, and the dried-blood-fresh-soil method. The nitrifying power was tested by the ammonium-sulphate-air-dry-soil method and the ammonium-sulphate-fresh-soil method. These methods were under investigation at the time of this study, and comparative tests of their efficiency have been reported in the work already referred to.[1]

Four samplings were made during the year—on August 9, August 19, October 7, and October 26. The variations in moisture content of the soils at the various dates were so slight that the differences observed could not be attributed to that factor, and the results of the determinations are not included here.

The crop yields were obtained from the plots as in the previous year.

The ammonification results appear in Tables VII, VIII, and IX, the nitrification results in Tables X and XI, and the crop yields are given in Table XII.

TABLE VII.—*Ammonification of dried blood in air-dry soil of rotation plots in 1912*

Plot No.	Quantity of ammonia (in milligrams of nitrogen).			
	Test 1.	Test 2.	Test 3.	Test 4.
601	148. 33	54. 93	124. 78	122. 42
603	157. 55	66. 31	130. 27	127. 92
605	170. 69	79. 77	138. 71	138. 71
608	172. 65	82. 40	141. 85	143. 42
610	168. 53	75. 73	136. 95	130. 67
902	151. 27	64. 15	125. 17	110. 09
904	161. 08	71. 61	131. 06	138. 21

TABLE VIII.—*Ammonification of dried blood in fresh soil of rotation plots in 1912*

Plot No.	Quantity of ammonia (in milligrams of nitrogen).			
	Test 1.	Test 2.	Test 3.	Test 4.
601	106. 34	68. 66	50. 81	54. 74
603	110. 66	80. 05	65. 14	62. 39
605	117. 32	86. 70	73. 77	71. 02
608	120. 87	88. 28	74. 02	74. 66
610	115. 95	78. 87	72. 59	71. 41
902	109. 67	73. 38	58. 80	62. 19
904	114. 14	82. 00	68. 28	69. 17

[1] Brown, P. E. Op. cit.

TABLE IX.—*Ammonification of casein on rotation plots in 1912*

Plot No.	Quantity of ammonia (in milligrams of nitrogen).			
	Test 1.	Test 2.	Test 3.	Test 4.
601	61. 80	64. 84	58. 66	55. 23
603	67. 30	71. 80	63. 13	66. 31
605	71. 61	76. 52	68. 47	69. 45
608	72. 39	79. 07	70. 63	72. 79
610	68. 67	73. 37	67. 29	69. 25
902	62. 78	69. 06	63. 18	61. 39
904	67. 10	73. 37	67. 10	68. 67

TABLE X.—*Nitrification of ammonium sulphate in the air-dry soil of rotation plots in 1912*

Plot No.	Quantity of nitrates (in milligrams of nitrogen).			
	Test 1.	Test 2.	Test 3.	Test 4.
601	10. 431	12. 443	8. 444	7. 252
603	13. 489	16. 751	12. 427	11. 353
605	15. 114	18. 941	15. 546	14. 557
608	15. 250	23. 931	16. 524	15. 259
610	14. 196	18. 110	15. 208	14. 733
902	12. 695	12. 893	9. 914	10. 076
904	14. 434	17. 410	14. 946	14. 666

TABLE XI.—*Nitrification of ammonium sulphate in the fresh soil of rotation plots in 1912*

Plot No.	Quantity of nitrates (in milligrams of nitrogen).			
	Test 1.	Test 2.	Test 3.	Test 4.
601	11. 944	15. 300	7. 183	6. 844
603	12. 728	16. 601	10. 695	9. 770
605	14. 682	22. 583	12. 462	12. 154
608	15. 520	25. 078	13. 784	14. 224
610	13. 559	18. 264	12. 233	13. 099
902	11. 960	15. 837	7. 789	10. 629
904	13. 060	17. 414	10. 981	13. 150

TABLE XII.—*The yield of corn on rotation plots in 1912*

Plot No.	Treatment.	Yield per acre.
		Bu.
601	Continuous corn	50. 25
603	Corn and oats	63. 12
605	Corn, oats, and clover	69. 00
608	Corn and oats; clover turned under	74. 00
610	Corn and oats; cowpeas turned under	68. 50
902	Corn and oats; rye turned under	50. 50
904	Corn, corn, oats, and clover	67. 50

If these results are examined, it is found that practically uniform agreement was secured with the various methods—i. e., the relative ammonifying powers of the soils were the same whether the dried-blood or the casein method was employed, and it made little difference whether the dried-blood-air-dry-soil method was employed or the dried-blood-fresh-soil method was used. Similarly, in the case of nitrification, the same relative results were obtained whether the air-dry-soil method or the fresh-soil method was employed. It is unnecessary, therefore, to consider the results individually, and comparisons will merely be made between the bacterial results and the crop yields.

The largest crop yield was obtained in this year on the plot under the 2-year rotation with clover turned under. Similarly, the greatest ammonifying power and the greatest nitrifying power were found in this soil. The soil under the 3-year rotation (corn, oats, and clover) was second in crop yield and in bacterial activities; the 2-year rotation with cowpeas as a green manure induced a slightly smaller crop yield and lower bacterial action; the 4-year rotation was still lower; the 2-year rotation (corn and oats) lower yet; the 2-year rotation with rye turned under gave a still smaller crop yield and lower bacterial action; and the continuous-crop plot was at the bottom of the list.

It is evident from these results that the ammonification and nitrification of nitrogenous organic material in soils and their crop-producing power are very closely related and that tests of the power of soils to produce ammonia or nitrates may be an indication of their crop-producing power, or at least of their relative crop-producing ability. Previous results are also confirmed regarding the similarity of the effects of soil treatment or ammonification and nitrification. Such need not always be the case, of course, as it is possible to conceive of conditions affecting the nitrifying organisms which do not similarly affect the ammonifiers, but it seems to be the case that in ordinary field conditions the two processes are quite similarly affected by treatment and probably only one process need be tested to gain some idea of the relative crop-producing power of soils.

TESTS ON ROTATION PLOTS IN 1913

The experiment on the same series of plots was continued in 1913, different individual plots being used for corn.

Three samplings were made during the season—on August 15, August 23, and August 26. Ammonification tests only were carried on, owing to the pressure of other work; and only one method, the casein-fresh-soil method, was employed. The crop yields were obtained as previously. Again, the moisture content of the soils at the different samplings varied so slightly that the differences may be considered negligible from the standpoint of the effects of treatment.

The results of the ammonification tests appear in Table XIII, and the crop yields are given in Table XIV.

TABLE XIII.—*Ammonification of casein on rotation plots in 1913*

Plot No.	Quantity of nitrogen.		
	Aug. 15.	Aug. 23.	Aug. 28.
	Mgm.	*Mgm.*	*Mgm.*
601	68. 38	60. 82	55. 67
602	71. 56	63. 47	59. 31
606	78. 74	69. 59	64. 05
607	74. 89	66. 35	63. 15
609	73. 53	64. 45	60. 52
901	75. 65	68. 25	63. 46
904	74. 28	65. 21	62. 97

TABLE XIV.—*Yields of corn on rotation plots in 1913*

Plot No.	Treatment.	Yield per acre.
		Bu.
601	Continuous corn	30. 0
602	2-year rotation: Corn and oats	53. 3
606	3-year rotation: Corn, oats, and clover	68. 0
607	2-year rotation: Corn and oats; clover turned under	64. 0
609	2-year rotation: Corn and oats; cowpeas turned under	60. 0
901	2-year rotation: Corn and oats; rye turned under	65. 3
904	4-year rotation: Corn, corn, oats, and clover	62. 6

Comparing the results, it is apparent that the indications of fertility given by the ammonification studies were borne out by the actual crop yields. The rank of the soils both in ammonifying power and in crop production was as follows:

Plot No.	Treatment.	Rank.
606	3-year rotation	1
901	2-year rotation; rye turned under	2
607	2-year rotation; clover turned under	3
904	4-year rotation	4
609	2-year rotation; cowpeas turned under	5
602	2-year rotation	6
601	Continuous corn	7

The results of these studies check those of previous years, therefore, and indicate that ammonification and crop production are very closely related and that the determinations of the ammonifying power of soils made during the growing season may show their relative crop-producing powers.

The plots in this series, as will be noted, ranked differently each year, both in crop yields and in bacterial activities, but it is not purposed to enter here upon a discussion of the reasons for such variations. The seasonal conditions, especially as regards rainfall, were undoubtedly of

prime importance. It will be noted, however, that the rotation of crops increased in every case both the crop yield and the bacterial activities. The use of green manure in the 2-year rotation sometimes proved more valuable than the 3-year rotation, and sometimes was of less value. This was probably due also to the moisture conditions. The point of importance here is, however, the fact that, regardless of seasonal conditions or of the effect on crops under particular conditions, bacterial activities and crop production were relatively similar.

TESTS ON MANURED PLOTS IN 1912

The manured plots were studied in 1912. Ammonification results were obtained by the casein-fresh-soil method, the dried-blood-air-dry-soil method, and the dried-blood-fresh-soil method; and nitrification tests were carried out by the ammonium-sulphate-air-dry-soil method and the ammonium-sulphate-fresh-soil method. Four samplings were made during the season—on August 2, August 15, August 22, and September 9. The moisture conditions in the soils varied so slightly that they could not be considered of significance, and they are not included here. Crop yields were secured, corn being grown on the plots as in the other series.

Complete data from these experiments have been reported in another place[1] and only summarized results are given here.

The ammonification results are given in Tables XV, XVI, and XVII, the nitrification results in Tables XVIII and XIX, and the crop yields in Table XX.

TABLE XV.—*Ammonification of dried blood in the fresh soil of manured plots in 1912*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
1004	60. 90	83. 97	73. 57	66. 71
1005	84. 76	92. 21	83. 97	70. 63
1006	86. 32	106. 34	98. 88	85. 54
1007	97. 90	109. 47	98. 88	84. 95
1008	86. 72	95. 74	87. 50	76. 91

TABLE XVI.—*Ammonification of casein on manured plots in 1912*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
1004	37. 87	68. 27	67. 49	51. 60
1005	46. 89	73. 57	72. 79	58. 86
1006	51. 79	77. 50	78. 87	60. 32
1007	51. 99	78. 48	79. 40	65. 72
1008	48. 78	75. 14	74. 75	60. 42

[1] Brown, P. E. Bacteriological studies of field soils. III. The effects of barnyard manure. Iowa Agr. Exp. Sta. Research Bul. 13, p. 421–448. 1913.

TABLE XVII.—*Ammonification of dried blood in the air-dry soil of manured plots in 1912*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
1004	80. 44	111. 83	106. 34	102. 81
1005	94. 76	117. 33	109. 47	117. 13
1006	100. 06	131. 25	122. 23	127. 92
1007	100. 85	137. 14	124. 00	133. 02
1008	95. 75	128. 90	113. 80	122. 62

TABLE XVIII.—*Nitrification of ammonium sulphate in the air-dry soil of manured plots in 1912*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
1004	8. 507	14. 794	12. 500	9. 211
1005	9. 326	15. 453	13. 693	10. 262
1006	10. 000	17. 710	14. 392	12. 593
1007	11. 655	18. 712	16. 401	12. 446
1008	10. 064	16. 696	14. 662	10. 444

TABLE XIX.—*Nitrification of ammonium sulphate in the fresh soil of manured plots in 1912*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
1004	5. 576	10. 946	10. 283	9. 141
1005	7. 259	12. 583	12. 543	10. 000
1006	8. 470	16. 733	14. 142	12. 698
1007	10. 282	18. 694	15. 641	13. 011
1008	8. 125	16. 104	12. 949	10. 528

TABLE XX.—*Yield of corn on manured plots in 1912*

Plot No.	Treatment.	Yield per acre.
		Bu.
1004	Check	50. 50
1005	8 tons of manure	77. 62
1006	12 tons of manure	86. 00
1007	16 tons of manure	87. 00
1008	20 tons of manure	81. 00

If the results secured in the ammonification tests are examined, it is seen that the effects of the manure were the same whatever method was employed. It is unnecessary, therefore, to consider the different results individually. Similarly in the case of nitrification, the fresh-soil and air-dry-soil methods yielded similar results, and general conclusions only need be drawn.

If the bacterial results as a whole are compared with the crop yields, it is found that there was exact agreement. Applications of manure increased the ammonifying and nitrifying powers of the soil, and the crop yield was also increased. Further gains in bacterial action and also in crop yields were obtained as the amount of manure applied was increased, but the maximum effect was obtained with the use of 16 tons of manure per acre. Beyond that point increasing the quantity of manure decreased both bacterial action and crop yields.

These results therefore check the previous observations that ammonification and nitrification tests may often run parallel. Previous results are also confirmed regarding the relation between crop yields and certain bacterial activities. Tests of the ammonifying power of soils or of their nitrifying powers apparently indicate quite accurately their crop-producing powers.

TESTS ON LIMED PLOTS IN 1911

The three plots in this series were sampled during 1911 on June 21, July 6, September 14, and October 24. Ammonification tests were made by the dried-blood and cottonseed-meal methods, nitrification by the ammonium-sulphate and dried-blood methods, and azofication by the mannite method. Crop yields were secured as in the other series studied. Complete results of these tests have been reported,[1] and only average results are given here.

The ammonification results appear in Tables XXI and XXII, the nitrification results in Tables XXIII and XXIV, the azofication results in Table XXV, and the crop yields in Table XXVI.

TABLE XXI.—*Ammonification of dried blood on limed plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
510	207. 17	206. 60	128. 06	129. 78
509	208. 12	207. 30	144. 51	140. 05
508	214. 13	235. 22	155. 59	149. 32

[1] Brown, P. E. Bacteriological studies of field soils. I. The effects of lime. Iowa Agr. Exp. Sta. Research Bul. 5, p. 187–210. 1912.

TABLE XXII.—*Ammonification of cottonseed meal on limed plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
510	131. 26	157. 22	126. 22	124. 32
509	132. 68	161. 06	141. 15	139. 28
508	142. 01	172. 58	151. 22	137. 90

TABLE XXIII.—*Nitrification of dried blood on limed plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
510	13. 745	27. 056	20. 579	14. 570
509	15. 844	33. 857	23. 247	18. 434
508	21. 911	39. 686	29. 376	22. 946

TABLE XXIV.—*Nitrification of ammonium sulphate on limed plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
510	8. 737	24. 987	14. 298	8. 762
509	10. 547	25. 475	20. 146	11. 743
508	14. 822	29. 034	24. 061	17. 890

TABLE XXV.—*Azofication tests on limed plots in 1911*

Plot No.	Quantity of nitrogen.			
	Test 1.	Test 2.	Test 3.	Test 4.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*
510	5. 52	2. 34	11. 89	11. 09
509	15. 07	16. 66	25. 41	27. 00
508	26. 21	30. 19	38. 93	37. 34

TABLE XXVI.—*Yield of corn on limed plots in 1911*

Plot No.	Treatment.	Yield per acre.
		Bu.
510	Check	52. 5
509	2 tons of lime	55. 0
508	3 tons of lime	55. 0

The ammonification results by the two methods employed were very similar, as also were the nitrification results; hence, these results need not be considered separately.

If the bacterial tests are compared with the crop yields, it is found that the lime increased ammonification, nitrification, and azofication in the soils, and the crop yield was similarly increased, the larger amount of lime bringing about the greater effect on the bacteria but exerting no further increasing effect on the crop grown.

These results as a whole therefore check those obtained on the plots under other methods of treament and show that bacterial transformations of nitrogenous compounds in the soil or, rather, the ability of soils to bring about the simplification of nitrogenous materials or the addition of nitrogen, may be considerably modified by various methods of soil treatment. Furthermore, they check previous results in showing that certain bacterial activities in the soil may be very closely related to the actual crop-producing power of the soil. The ammonifying power of soils, their nitrifying power, or even, in certain cases, their azofying power may therefore indicate the crop-producing power of soils or, at least, their relative crop-producing power.

CONCLUSIONS

(1) These experiments as a whole represent a line of investigation in soil bacteriology which it is believed will ultimately place the subject on a more practical basis—a basis which will permit the direct application of the results obtained to the solution of soil-fertility problems.

(2) The relations between the bacterial activities studied and the actual crop yields on these plots have proved so striking and so consistent that it was felt that accidental coincidence had been practically eliminated and the results might be considered to give a strong indication that certain bacterial activities in field soils are very closely associated with crop yields.

(3) Furthermore, the tentative conclusion presents itself that tests of such bacterial activities in the laboratory may indicate quite accurately the crop-producing power of a soil or, at least, the relative crop-producing power of several soils.

(4) If, further, more exhaustive tests confirm these preliminary observations, it may be possible to secure advance information regarding the crop-producing power of soils by means of laboratory tests of bacterial action in those soils.

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